Anthelmintic resistance in cattle – status and new alternatives for control

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Outline

1. Background
2. Anthelmintic resistance
3. Feeding: esparsette & condensed tannins
4. Feeding: chicory & sesquiterpenlactones
5. Conclusions & perspectives
Gastrointestinal nematodes (GIN) (løbetarmorm)

- Most pathogenic and prevalent species in cattle:
  - *Ostertagia ostertagi* (abomasum)
  - *Cooperia oncophora* (small intestine)

Life cycle of GIN
Control of gastrointestinal nematodes

Use of anthelmintic drugs in Danish cattle 2010-2014:

- The heavy reliance on drugs increases the risk of anthelmintic drug resistance, particularly ivermectins
- Increasing problems worldwide in cattle

Source: VetStat
Control of gastrointestinal nematodes

Objectives:
- examine selected Danish cattle farms for anthelmintic resistance (AR) against ivermectin
- investigate alternative options for control by means of feeding specialized (bioactive) crops
Faecal egg count reduction test (FECRT)

- N=120 first-grazing season calves (6 farms)
- Animals stratified by egg count and randomly allocated into:
  - **Treatment group (IVM):** 0.2 mg IVM s.c./kg LW (n= 10/farm)
  - **Control group (CTL):** Untreated (n=10/farm)
- Egg counts at day of treatment (D0) and 14 days post treatment (D14)
  - **FECR% (arit. mean) = 100 \times (1 – [IVM D14/IVM D0]); 95% C.I.**
  - **Markov chain Monte Carlo (MCMC) model = 95% C.I.**
- **Interpretation:**
  i) Efficacious, when mean FECR% and upper CI ≥ 95% and lower CI ≥ 90%;
  ii) Reduced efficacy (AR), when mean FECR% and upper CI < 95% and lower CI < 90%;
  iii) Inconclusive, when none of the above conditions were met.

(Coles et al. 1992; Denwood et al. 2010; Lyndal-Murphy et al 2014)
### FECRT: results from six Danish cattle farms

<table>
<thead>
<tr>
<th></th>
<th>Farm #1 (Beef, conv.)</th>
<th>Farm #2 (Dairy, org.)</th>
<th>Farm #3 (Dairy, conv.)</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
<td>IVM</td>
<td>CTL</td>
<td>IVM</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td><strong>WAAVP</strong></td>
<td>86&lt;sup&gt;R&lt;/sup&gt; [66–94]</td>
<td>82&lt;sup&gt;R&lt;/sup&gt; [47–94]</td>
<td>92&lt;sup&gt;i&lt;/sup&gt; [30–99]</td>
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<tr>
<td><strong>MCMC</strong></td>
<td>87&lt;sup&gt;R&lt;/sup&gt; [81–93]</td>
<td>83&lt;sup&gt;R&lt;/sup&gt; [72–92]</td>
<td>90&lt;sup&gt;i&lt;/sup&gt; [62–98]</td>
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</table>

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<tr>
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<th>Farm #4 (Dairy, org.)</th>
<th>Farm #5 (Beef, org.)</th>
<th>Farm #6 (Dairy, org.)</th>
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</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td>IVM</td>
<td>CTL</td>
<td>IVM</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
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<td>83&lt;sup&gt;i&lt;/sup&gt; [-50–98]</td>
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<td><strong>MCMC</strong></td>
<td>81&lt;sup&gt;R&lt;/sup&gt; [50–94]</td>
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<td>81&lt;sup&gt;i&lt;/sup&gt; [25–99]</td>
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</table>

- predominantly *Cooperia* post-treatment
Nutritional manipulation/bioactive feeds

- Bioactive crops may be incorporated in the feed on stable or in the pasture

- So-called bioactive crops contain a huge range of **Plant Secondary Metabolites (PSM):** alkaloids, phenolics (tannins), terpenoids etc.

- Some used in traditional, ethnoveterinary medicine

- Scientific validation needed (Hoste et al, 2015)

An example:
Condensed tannins are found in
- Sainfoin (esparsette) (1-8%)
- Birdsfoot trefoil (kællingetand)
- Several berries
- Red wine!!

![Illustration of Procyanidin (PC) and Prodelphinidin (PD) structures]
**In vitro** anti-parasitic effects of extracts

Larval Feeding Inhibition Assay LFIA (free-living stage)

*L1*  
*E. coli* labelled with FITC

- incubation 2 hours  
- incubation 18 hours

Fed larvae  
Unfed larvae

Chicory cv. Spadona rich in **sesquiterpen-lactones (SL)**

Chicory cv. Puna II

- similar high efficacy with sainfoin extracts
Sainfoin (esparsette): *in vivo* study

**SAINFOIN**
- Dehydrated pelleted sainfoin (Perly)

**ANIMALS**
- 2.5–4.5 month-old Jersey calves

**GROUPS AND DIETS**
- **Control group** (*n*=6)
  - Concentrate (50-65%) + ryegrass-clover hay
- **Sainfoin Group** (*n*=9)
  - Pellets sainfoin (90%) + ryegrass-clover hay

**TIMELINE**
- 16 days: isoprotein-energy diets
- 42 days: Infection
  - 10,000 L3 *O. ostertagi*
  - 66,000 L3 *C. oncophora*
- Faecal Egg Counts (FEC) (3× week)
- Adult worms
  - (burden; sex ratio; fecundity)
Sainfoin: *in vivo* study - results

Effect on adult worms but no effect on egg counts:

<table>
<thead>
<tr>
<th>Adult nematodes</th>
<th>Group</th>
<th>Worm burden</th>
<th>♂ (%)</th>
<th>♀ fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ostertagia ostertagi</em></td>
<td>CO</td>
<td>2,715 ± 894</td>
<td>44 ± 6</td>
<td>41 ± 09</td>
</tr>
<tr>
<td></td>
<td>SF</td>
<td>1,331 ± 947*</td>
<td>42 ± 7</td>
<td>43 ± 12</td>
</tr>
<tr>
<td><em>Cooperia oncophora</em></td>
<td>CO</td>
<td>22,447 ± 17,639</td>
<td>34 ± 15</td>
<td>53 ± 45</td>
</tr>
<tr>
<td></td>
<td>SF</td>
<td>19,664 ± 22,496</td>
<td>29 ± 23</td>
<td>40 ± 36</td>
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</tbody>
</table>

Scanning electron microscopy to visualise cuticle damage on adults of *O. ostertag*
Sainfoin: analysis of Condensed Tannins (CT) in different gut sections

CT content (% of dry matter)
Thiolysis coupled with LC-MS (n=8)

- Little or no absorption of CT
- Accessibility of CT important for anthelmintic activity
- Thiolysis method good indicator of anthelmintic activity

RU: rumen, AB: abomasum
SI: small intestine, FE: faeces

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SI: small intestine, FE: faeces

-50%

O. ostertagi

No effect
C. oncophora
Chicory (SL-rich): grazing study - results

N = 20 calves
Chicory: 90% of pasture DM (11% CP)
Ctrl: clover grass (16% CP)

- no effect on *Cooperia* in the small intestine!

Geo mean FEC adjusted for faecal DM (FECDM) in chicory and control groups (95% CI)

Mean *O. ostertagi* adult counts in chicory and control groups (95% CI)
Conclusions & Perspectives

- AR present in Danish cattle farms (3 out of 6 farms)
  ⇒ so far mainly of academic interest
  ⇒ no reported drug failures
  ⇒ implications for control?

- Sainfoin and chicory are potent against *O. ostertagi* in cattle (*in vivo*)
  ⇒ *Ostertagia* more pathogenic and found in older animals

- How to tackle the lack of *in vivo* effect against *Cooperia*?

- Both content and type/structure of CT and SL matter for AH activity
  ⇒ Selection of right species/cultivar of crop or improved breeding

- Mechanisms of action remain to be elucidated
  ⇒ New FTP project for CHICORY and SL just initiated (Plants & Parasites)
Acknowledgements of funding bodies:

CARES: Coping with Anthelmintic RESistance in ruminants

Ministry of Food, Agriculture and Fisheries of Denmark