Epidemiology and diagnosis of bovine cysticercosis

Peter Deplazes

Prevalence

<table>
<thead>
<tr>
<th>Year</th>
<th>Bull</th>
<th>All</th>
<th>#</th>
<th>#</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>1.0</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>1.63%</td>
</tr>
<tr>
<td>1991</td>
<td>1.5</td>
<td>8</td>
<td>1</td>
<td>1.33%</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>1.6</td>
<td>8</td>
<td>3</td>
<td>1.53%</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>1.6</td>
<td>8</td>
<td>2</td>
<td>1.65%</td>
<td></td>
</tr>
<tr>
<td>1994</td>
<td>1.4</td>
<td>8</td>
<td>2</td>
<td>1.65%</td>
<td></td>
</tr>
</tbody>
</table>

Cysticercosis in cattle: Abattoir records in Italy

<table>
<thead>
<tr>
<th>Significance?</th>
<th>EU approved abattoirs in Switzerland</th>
<th>Heter</th>
<th>Bull</th>
<th>Ox</th>
<th>Cow</th>
</tr>
</thead>
<tbody>
<tr>
<td># 1</td>
<td>3</td>
<td>20.33</td>
<td>0.24</td>
<td>1.46%</td>
<td></td>
</tr>
<tr>
<td># 2</td>
<td>4</td>
<td>52.38</td>
<td>0.13</td>
<td>1.30%</td>
<td></td>
</tr>
<tr>
<td># 3</td>
<td>5</td>
<td>78.98</td>
<td>0.49</td>
<td>0.49%</td>
<td></td>
</tr>
<tr>
<td># 4</td>
<td>10</td>
<td>17.35</td>
<td>0.32</td>
<td>0.45%</td>
<td></td>
</tr>
<tr>
<td># 5</td>
<td>2</td>
<td>11.90</td>
<td>0.18</td>
<td>0.99%</td>
<td></td>
</tr>
</tbody>
</table>
**Discussion**

Known parasite transmission

Insufficient diagnostic tools

Doubts in strategy for control

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**Analyses of effluent from a sewage purification plant**

<table>
<thead>
<tr>
<th>Date</th>
<th>Total amount</th>
<th>Result Microscope</th>
<th>Result multiplex PCR*</th>
<th>Sequencing results</th>
</tr>
</thead>
<tbody>
<tr>
<td>03.10.06</td>
<td>200 l</td>
<td>-</td>
<td>267 bp</td>
<td>T. saginata</td>
</tr>
<tr>
<td>09.10.06</td>
<td>300 l</td>
<td>-</td>
<td>267 bp</td>
<td>T. saginata</td>
</tr>
<tr>
<td>11.10.06</td>
<td>200 l</td>
<td>-</td>
<td>267 bp</td>
<td>T. saginata</td>
</tr>
<tr>
<td>13.10.06</td>
<td>200 l</td>
<td>2-3 taenid eggs</td>
<td>267 bp 395 bp</td>
<td>n.d.</td>
</tr>
<tr>
<td>18.10.06</td>
<td>250 l</td>
<td>-</td>
<td>267 bp</td>
<td>Diphyllobothrium spp.</td>
</tr>
<tr>
<td>23.10.06</td>
<td>200 l</td>
<td>-</td>
<td>267 bp</td>
<td>T. saginata</td>
</tr>
<tr>
<td>25.10.06</td>
<td>200 l</td>
<td>eggs</td>
<td>267 bp 395 bp</td>
<td>Hymenolepsis spp. n.d.</td>
</tr>
</tbody>
</table>

*Trachsel et al., 2007.

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**Transmission in Switzerland:**

- Most farms are family-run
- Average roughage area per farm: 22.3 ha
- Herd size per farm: 36.2 cattle units
- 60% without employees, 40% with an average of 3.2 employees

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**Cysticercosis in cattle: financial losses for farmers in Switzerland**

<table>
<thead>
<tr>
<th>Losses for farmers</th>
<th>Cow</th>
<th>Beef cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total loss per animal (no insurance)</td>
<td>€ 465.-</td>
<td>€ 685.-</td>
</tr>
<tr>
<td>Total loss per animal (with insurance)</td>
<td>€ 238.-</td>
<td>€ 498.-</td>
</tr>
<tr>
<td>% deduction (from normal meat price per kilo)</td>
<td>45.3 %</td>
<td>51.0 %</td>
</tr>
</tbody>
</table>

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**Risk analysis for cysticercosis in cattle: inclusion criteria on farm level**

**119 positive farms**

Animals at least 9 months on the farm
So far no records of cysticercosis on the farm

**66 negative farms**

Confirmed infection: visual PCR (PCR as described: González et al., 2000)
“homemade” infection:
animals on the same farm for at least 9 months with viable cysticerci
24 months with calcified lesions

Questioning during farm visit or by phone interview
Stool examination for *T. saginata* infections

317 samples from 49 farms (41% of the positive farms)

Coproscopy: all negative

Coproantigen-ELISA (Deplazes et al. 1991):
308 samples analysed
- 266 negative (86.3%)
- 35 not interpretable (11.4%)
- 7 positive (2.3%)

Egg isolation by filtration from coproantigen-positive samples (Mathis et al. 1996)
- all negative

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Definitions of factors included in the risk analysis for bovine cysticercosis

<table>
<thead>
<tr>
<th>Variable on farm level</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd size</td>
<td>Cattle units (1 unit = 500 kg live weight)</td>
</tr>
<tr>
<td>Animal category</td>
<td>Cow, beef cattle (each conventional or mother-cow-husbandry), heifer</td>
</tr>
<tr>
<td>Roughage area</td>
<td>Area administered where cattle graze and where grass is harvested (ha)</td>
</tr>
<tr>
<td>Grazing time</td>
<td>Hours per year animal spends on pasture</td>
</tr>
<tr>
<td>Transhumance</td>
<td>Grazing on alpine pastures at least once</td>
</tr>
<tr>
<td>Streams</td>
<td>Direct or indirect (flooded pastures or fields) access to water from streams</td>
</tr>
<tr>
<td>Fresh grass</td>
<td>Feeding grass in the stable without storage</td>
</tr>
<tr>
<td>Purchased roughage</td>
<td>Hay or silage (grass/corn) also from external production</td>
</tr>
<tr>
<td>External manure</td>
<td>Manure from other farms spread on land</td>
</tr>
<tr>
<td>Domestic sewage</td>
<td>Waste water from households connected to effluent pond</td>
</tr>
</tbody>
</table>

Variable for farm surrounding Definition

| Employees | Employee(e)s working on farm within past five years |
| Visitors  | Organised activities on farm (events, open days and other hospitality activities), horse pension |

Swiss federal railways: Around 60% of wagons have an open toilet system

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Risk factors in analysis based on 19 variables tested

- Herd size
- Organic or conventional contact streams
- Feeding surface
- Grazing time
- Transhumance
- Purchased roughage
- Grass feeding
- External manure
- Domestic sewage
- Employees
- Visitors
- Leisure activities
- Military activities
- Parking places
- Wild camping
- Picnic place/viewpoint
- Railway line

Risk factors in analysis based on 19 variables tested

- 1. Pre-selection of factors
  - Stepwise backwards selection with Akaike information criterion (AIC; Software R)
- 2. Final selection (as AIC is very conservative)
  - Likelihood-ratio test
Significant results of the logistic regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Present (% of the farms)</th>
<th>Odds ratio</th>
<th>95% C.I.</th>
<th>p-value (LRT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control farms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Railway line</td>
<td>7.58</td>
<td>22.69</td>
<td>3.72</td>
<td>1.38-11.91</td>
</tr>
<tr>
<td>Leisure activities</td>
<td>4.55</td>
<td>13.45</td>
<td>3.58</td>
<td>1.05-16.59</td>
</tr>
<tr>
<td>Car park</td>
<td>9.09</td>
<td>26.05</td>
<td>9.05</td>
<td>2.00-8.91</td>
</tr>
<tr>
<td>Purchased roughage</td>
<td>18.18</td>
<td>33.61</td>
<td>2.99</td>
<td>1.37-6.49</td>
</tr>
<tr>
<td>Visitors</td>
<td>7.58</td>
<td>21.01</td>
<td>2.87</td>
<td>1.06-9.22</td>
</tr>
</tbody>
</table>

a) LRT: Likelihood ratio test based on the final model compared to the one without the relevant variable
b) 95% confidence intervals

Discussion

Direct transmission on farms could not be demonstrated in this study!

Attempts to diversify the income in the Swiss agriculture, including tourist activities and farm events, might contribute to new risks for cysticercosis.

“Old” risks such as railway lines crossing farm grounds should in a long term be of minor importance.

New strategies for control have to be considered.

Epidemiology EU


Most likely route of T. saginata introduction:
- Questionnaire (based on the EFSA risk assessment)
- on each affected farm (N = 67)
- 5 risk factors included (water supply for animals, feed, pastures, personnel and other routes)
- 82% response

Results:
- Water supply for animals was the route with the highest score in 41.8% of the cases (23 farms)
- Feed in 9.1% (5 farms)
- other routes in 12.7% (7 farms)

Epidemiology EU

- Questionnaire to 1500 farms (750 positive and 750 negative)
- 56.1% response
- 28 factors included

Results:
- province
- number of cattle slaughtered in 2003
- Flooding of pastures
- free access of cattle to surface water
- Proximity to waste water effluent within a range of 200m around the farm

Diagnosis at meat inspection

25. The official veterinarian must examine, in particular:
(d) the pericardium and the heart, the latter being indeed lengthwise as to open the ventricles
26. In addition, the official veterinarian must systematically carry out: A. An investigation for cysticercosis: (a) in bovine animals over six weeks old, at the level of:
- the tongue, of which the musculature must be incised lengthwise on the lower surface, without damaging the organ excessively;
- the oesophagus, which must be freed from the trachea;
- the heart, which, in addition to the incision provided for in paragraph 25 (d), must be split from two opposite points from the auricles to the apex of the external and internal musculature, which shall be incised along two planes parallel to the mamillary from its lower edge to its upper muscular insertion;
- the diaphragm, the muscular part of which must be freed from the spongy part;
- the muscular surfaces of the carcass which are directly visible;

Prevention / Meat inspection:

Cutting musculature:
- inner & exterior cheek muscles
- heart

Visual examination:
Sensitivity of routine meat inspection:

**Diagnosis at meat inspection: an old story**

Drewart et al. (1967). “Comparison of prescribed US inspection procedures for bovine cysticercosis with more extensive examinations of the predication sites showed that the prescribed procedures did not detect infection in at least 26% of the carcasses.”

Geerts et al., (1980). “Hearts of 100 slaughtered cattle, approved fit for human consumption at meat inspection, [...] in 25 per cent of the animals one or more caseous or calcified cysts were recovered.”

Walder & Koske, (1980). [...]. At dissection 75.9% were found infected. However, only 38.3% of the infected carcasses were detected at meat inspection.”

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**Diagnosis at meat inspection**

Sensitivity of routine meat inspection:

Dörny et al. (2000). “[...] the prevalence of bovine cysticercosis was more than 10 times higher with the antigen detection ELISA than by classical meat inspection.”

Underestimation of the prevalence by a factor 3 to 10

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**Diagnosis at meat inspection**

Sensitivity of routine meat inspection:

Estimation by Walther & Koske (1980) of the sensitivity of detecting cysticerci at routine post mortem meat inspection:

<table>
<thead>
<tr>
<th>Animals with</th>
<th>% of animal in which cysticerci were detected by routine meat inspection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10 cysts</td>
<td>27%</td>
</tr>
<tr>
<td>11-20 cysts</td>
<td>42.9%</td>
</tr>
<tr>
<td>20 cysts</td>
<td>77.8%</td>
</tr>
</tbody>
</table>

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**Predilection sites**

Lopes et al., (2010): “Preferential infection sites of Cysticercus bovis in cattle experimentally infected with Taenia saginata eggs”

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**“Increased sensitivity of routine meat inspection”**

Methods:

- 6 additional muscle cuts (heart)
- 3 EU-approved abattoirs in Switzerland (N=1,088)
- routine diagnosis performed at meat inspection
- microscopical and molecular confirmation of positive abattoir result

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High as confirmed by PCR even in calcified lesions:

**Diagnosis at meat inspection: specificity**

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10.06.2010
“Increased sensitivity of routine meat inspection”

Results:
- Investigated carcasses N = 1088
- Positive by routine meat inspection 20 (1.8 %)
- Positive by additional cuts 29 (2.7 %)
- Total positive cases 49 (4.5 %)

<table>
<thead>
<tr>
<th>No. additional cut</th>
<th>Positive/total positive (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20/1088 (1.8 %)</td>
</tr>
<tr>
<td>1</td>
<td>29/1088 (2.7 %)</td>
</tr>
<tr>
<td>2</td>
<td>49/1088 (4.5 %)</td>
</tr>
</tbody>
</table>

“Increased sensitivity of routine meat inspection”

Results:
- 103 cysticerci were located
- 91.8% of the cases had a single cyst
- by total heart dissection, 8 animals also had viable cysts next to degenerated ones.
- routinely performed meat inspection of the heart:
  - 80% (335) cysticerci of viable infection status
- additional examination of the heart:
  - 25.8% (8/31) cysticerci of viable infection status

“Evaluation of diagnostic tools for bovine cysticercosis”

Detection of circulating Ag:
- MoAb: B156C10A8/861H1A1 (anti-E/S)
- Van Kerckhoven et al. (1998)
  - Sensitivity: 92%, Specificity: 98.7% > 50 cysts per infected animal
- Dorny et al. (2000)
  - Sensitivity: 12.8%, Specificity: 98.7% < 50 cysts per infected animal

“Alternative tools for the diagnosis of bovine cysticercosis”

- Detection of specific antigens in serum
- Detection of specific antibodies in serum or meat juice

“Evaluation of diagnostic tools for bovine cysticercosis”

Peptides for detection of specific Ab:
- Characterized by Bonay et al., (2001)
  - HP6-2 (major secreted antigen of activated onchospheres)
  - Ts45S-10 (1. ové 45S homologue protective antigen)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Se</th>
<th>Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP6-2</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td>Ts45S-10</td>
<td>100%</td>
<td>97%</td>
</tr>
</tbody>
</table>

CD group:
100 young calves reared under hygienic conditions
Positive animals: 3 experimentally infected animals
Evaluation of serological tools for bovine cysticercosis: Antigens

Antigen-detecting tests: ELISA

In preparation, collaboration with P. Dowy

Evaluation of serological tools for bovine cysticercosis: Samples

Serum and meatjuice samples:
- 49 healthy cattle (serum and meat-juice)
- 51 naturally infected samples
- 9 experimentally infected serum samples
- 61 cattle without liver-fluke
- 47 cattle with Fasciola hepatica infection
- 168 cattle with Dicrocoelium dendriticum infection

Factors influencing transmission and control

Asymptomatic infection: few definitive hosts can contaminate large areas
Survival in the environment
Meet inspection with low sensitivity
Vaccination commercially not available
Chemotherapy expensive, will not eliminate calcified lesions
Risk factors for bovine infection: variable

Evaluation of serological tools for bovine cysticercosis: results

<table>
<thead>
<tr>
<th>Test</th>
<th>Healthy (n=197)</th>
<th>Infected (n=67)</th>
<th>Cattle/Insects</th>
<th>Literature Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide HP6-2</td>
<td>88.8</td>
<td>8.8</td>
<td>100</td>
<td>98% ± 5%</td>
</tr>
<tr>
<td>Peptide Ts5S-10</td>
<td>70.6</td>
<td>40</td>
<td>100</td>
<td>87% ± 5%</td>
</tr>
<tr>
<td>Crude cysticercus extract (Cca)</td>
<td>53.9</td>
<td>38.5</td>
<td>100</td>
<td>84% ± 6%</td>
</tr>
<tr>
<td>Larval E/S</td>
<td>68.9</td>
<td>76.6</td>
<td>97.6</td>
<td>90% ± 4%</td>
</tr>
</tbody>
</table>

Canadian control system

Thank you for your attention!