Associations between delayed type hypersensitivity to purified protein derivative and MAP-antibody ELISA in Irish dairy cows

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Mycobacteria

- *Mycobacterium bovis*- bovine tuberculosis (bTB)
- Intradermal skin test
- Purified protein derivative (PPD)
  - Avian
  - Bovine
- Single intradermal cervical comparative test (SICCT)
- SICCT: skin thickness- mm (0hrs + 72hrs)
- Delayed type hypersensitivity response (DTH)
- Ireland – comprehensive bTB eradication programme
  - All bovines over 6 weeks
  - Minimum one test per year
Johne’s Disease
*Mycobacterium avium* subspecies *paratuberculosis* (MAP)

**Clinical signs include:**
- Decreased performance
- Weight loss
- Diarrhoea
- Bottle jaw
- Eventual death

- Usually start between 2 to 6 years

**Infection:**
- Infection usually occurs as calves
- Ingestion of MAP infected
  - Faeces
  - Colostrum
  - Milk

- Treatment ineffective – Break cycle of transmission
  - Identify and Remove Infected animals

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Introduction

• Johne’s disease (JD)
  • ELISA
  • PCR
  • Culture
  • Sensitivity & Specificity
• Intradermal testing
  • aPPD, johnin, SICCT
• OIE

Aim

• DTH (skin reaction) to aPPD or bPPD → increase in MAP ELISA

• Potentially identify research areas aid MAP diagnosis
Background

• Examine impact of SICCT (PPD administration) on MAP ELISA

  • Aim: Identify suitable time MAP diagnostics post SICCT
  • Milk and serum samples pre & post SICCT
  • ELISA- IDvet MAP Antibody Test Kit

Statistical analysis

• ELISA results - categorical and continuous variable
Herd prevalence of ELISA positives

Aim

- DTH (skin reaction) to aPPD or bPPD → increase in MAP

ELISA

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Materials and Methods

- 139-cow spring-calving
- 54%-HF 46%-JX
- Clinical case JD 2013- confirmed Post Mortem (PM)
- ELISA negative herd 2014
- 3 culture positive cows 2015
Materials and Methods

• Statutory SICCT

• SICCT: skin thickness- mm (0hrs + 72hrs)

• DTH categories
  • DTH at both aPPD and bPPD sites
  • DTH at one PPD administration site only
  • No DTH
Materials and Methods

- Blood samples
  - Pre SICCT
  - Post SICCT: Day 10, 16, 42, 57, and 91
- ELISA
  - IDvet MAP Antibody Test Kit
  - S/P ratio ≥ 70: Positive

Statistical Analysis

- Generalised Estimating Equations
- ELISA results: categorical (+/-) & continuous variable (S/P)
- DTH results: categorical & continuous (skin thickness mm’s)
- Independent Variables
  - Time point, breed & parity
DTH Results

- No TB reactor (i.e. no bPPD 4mm > aPPD)
- DTH both PPD sites- 35
- DTH aPPD only- 54
- No DTH- 50
MAP ELISA Results

- ELISA negative pre-SICCT
- Day 10 post-SICCT 30% positive
Associations between DTH response (mm), at each PPD administration site, and MAP ELISA S/P ratio post SICCT

<table>
<thead>
<tr>
<th>Time</th>
<th>Site of PPD inoculation</th>
<th>Coefficient (a)</th>
<th>(P) Value</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10 vs. Pre- SICCT</td>
<td>Avian</td>
<td>4.9</td>
<td>&lt;0.001</td>
<td>3.0, 6.7</td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>7.6</td>
<td>&lt;0.001</td>
<td>3.9, 11.3</td>
</tr>
<tr>
<td>Day 16 vs. Pre- SICCT</td>
<td>Avian</td>
<td>5.3</td>
<td>&lt;0.001</td>
<td>3.0, 7.8</td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>5.2</td>
<td>&lt;0.001</td>
<td>0.3, 10.1</td>
</tr>
</tbody>
</table>

\(a\)Coefficient: represents the increase in MAP ELISA S/P ratio for every 1mm increase in DTH skin thickness response

DTH aPPD & bPPD : O.R. 3.9 times more likely to test MAP ELISA positive
**Box plots:** Differences in MAP S/P ratios between DTH categories

- **MAP ELISA S/P Ratio**
- **Pre-SICCT, Day 10, Day 16, Day 42, Day 57, Day 91**
- **Categories:**
  - aPPD & bPPD DTH
  - aPPD only DTH
  - No DTH

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Discussion

Increased Sensitivity v’s Decreased Specificity?

- Increased Sensitivity
  - MAP + show DTH at both PPD administration sites
  - aPPD & bPPD DTH cows > O.R.MAP+ post SICCT

- Decreased Specificity
  - Culture positive animals: Not + at aPPD & bPPD sites
Conclusion

• Association between PPD hypersensitivity and MAP ELISA

• Improved sensitivity or decreased specificity?

• Suggestive of decreased specificity
Questions?