Accuracy of commercially available paratuberculosis ELISA kits and an in-house ELISA on bovine milk samples

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Introduction

The antibody produced during *Mycobacterium paratuberculosis* infection in cattle is present in both serum and milk. However, milk is more conveniently collected than serum and thus is a more cost-effective clinical sample. The two goals of the study were (1) to evaluate the specificity of milk ELISAs and (2) to correlate the results for serum vs. milk for each of four commercial paratuberculosis ELISA kits and an “in-house” ELISA.

To estimate milk ELISA specificity (goal 1), serum and milk samples were tested from non-infected adult Holstein cows (n=213) resident in herds that tested negative for >4 years and qualify for Level 4 in the Voluntary Bovine Johne’s Disease Control Program.

For goal 2, results for matched serum and milk samples per ELISA were correlated for each of 82 JD-suspects (i.e. cattle previously fecal culture or IDEXX ELISA positive). These cattle were from *Mycobacterium paratuberculosis*-infected dairy herds.

Methods

ELISAs

- IDEXX, Prionics (Parachek), ID-VET ELISAs performed per manufacturer’s specifications
- JTC-ELISA per “in-house” specifications
- In the four ELISAs, the serum and milk samples were pre-absorbed with *Mycobacterium phlei* antigens. The absorption protocol for the fourth commercial ELISA (Antel), if any, is not known.

Samples: individual serum & milk collected at the same time

- Previously test-positive (fecal culture or IDEXX ELISA) Holstein cows in confirmed *M. paratuberculosis*-infected herds (n=82)
- Previously test-negative (fecal culture or IDEXX ELISA) Holstein cows resident in status Level 4 herds (n=213)

Data analysis:

- Proportion of serum ELISA positive JD-suspects that are milk ELISA positive using the same ELISA kit
- Specificity, i.e. percentage of noninfected cows testing negative.
- ROC curves (Prism® ver 4.03; GraphPad Software, Inc.)
- Area under the ROC curve
- Agreement & kappa for comparison of serum vs. milk sample results with the same ELISA kit.
- Linear regression of serum and milk ELISA results

Results

- To estimate milk ELISA specificity (goal 1), serum and milk samples were tested from non-infected adult Holstein cows (n=213) resident in herds that tested negative for >4 years and qualify for Level 4 in the Voluntary Bovine Johne’s Disease Control Program.
- For goal 2, results for matched serum and milk samples per ELISA were correlated for each of 82 JD-suspects (i.e. cattle previously fecal culture or IDEXX ELISA positive). These cattle were from *Mycobacterium paratuberculosis*-infected dairy herds.

Conclusions

Some ELISA kits for bovine paratuberculosis designed for use on serum samples can effectively be used with individual bovine milk samples with limited loss in overall diagnostic accuracy. ELISA kits from Prionics, ID-VET, and an “in-house” ELISA from UW (JTC-ELISA) performed comparably well.

Summary Comparison Statistics

<table>
<thead>
<tr>
<th></th>
<th>IDEXX</th>
<th>Prionics</th>
<th>ID-VET</th>
<th>JTC-ELISA</th>
<th>AntelBio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Milk ELISA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive rate*</td>
<td>14.1%</td>
<td>88.0%</td>
<td>72.5%</td>
<td>91.1%</td>
<td>50.3%*</td>
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<tr>
<td>Specificity</td>
<td>99.5%</td>
<td>99.5%</td>
<td>99.0%</td>
<td>99.4%</td>
<td>91.9%</td>
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<tr>
<td>ROC</td>
<td>0.748</td>
<td>0.911</td>
<td>0.682</td>
<td>0.561</td>
<td>0.702</td>
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<tr>
<td>Serum/Milk Agreement</td>
<td>92.9%</td>
<td>92.7%</td>
<td>92.7%</td>
<td>92.9%</td>
<td>92.6%</td>
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<tr>
<td>Kappa</td>
<td>0.07</td>
<td>0.65</td>
<td>0.65</td>
<td>0.67</td>
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<tr>
<td>Regression r²</td>
<td>0.261</td>
<td>0.704</td>
<td>0.671</td>
<td>0.779</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage of serum ELISA-pos cows detected by milk ELISA (using the same ELISA kit), except for AntelBio where value is percentage of all JD-suspects as sera not tested by AntelBio.