Bovine Abortions and Stillbirths in Denmark 2015 to 2017

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Infections are the most common cause of bovine abortion. Here we report recent diagnostic findings in bovine abortion material from Denmark, a country with a large dairy sector and high animal health standards. This study was conducted in order to gain in-depth knowledge on infectious causes of abortions i.e. to identify and localize infectious agents in placental and foetal tissues. The cultivation-independent methods Fluorescence in situ hybridization (FISH) and second generation sequencing were applied additionally to routine histopathology and bacterial cultivation.

### STUDY POPULATION

- Danish Holstein 62%
- Danish Jersey 13%
- Crossbreed 9%
- Danish Red 7%

0.8% of reported abortions during study period.

Figure 1. The study population consisted predominantly of dairy cows (5% beef) from mainly conventional farms (9% organic) and originated from across the country matching the geographical distribution of dairy farms in Denmark (map displaying dairy farms as grey dots and abortion submitting farms as red dots). The majority of the abortions took place during mid to late gestation.

### SAMPLE MATERIAL

- Oral route: placental interface, lung, kidney
- Systemic route: brain, heart

Figure 2. Organs collected at necropsy for histological and molecular analyses. Organs were chosen based on known predeliction sites for abortogenic infections (e.g. neosporosis in brain, liver, heart; Leptospira interrogans in kidney) and related to potential ports of entry into the foetus. Abortion material was submitted to DTU Vet for routine abortion diagnostics from January 2015 until June 2017.

### RESULTS

#### BRUCELLA ABORTUS CULTIVATION

All foetal organ pools were negative for Brucella abortus.

#### HISTOPATHOLOGICAL SCREENING FOR NEOSPORA CANTAN

Neosporosis was diagnosed in 30 out of 162 abortions (19%) based on findings in HE stained tissue sections of brain, heart, and liver.

ELISA

In 90% of the cases, a blood sample of the dam was submitted. All samples were negative for maternal BVDV antibodies.

### CONCLUSIONS

- **Neosporosis** was the most frequently diagnosed infection.
- **No epizootic abortifacients** were found on study population level, however, due to very few abortions submitted per herd, no conclusions can be drawn on herd level.
- **Fungi** seem to play a minor role as abortogenic agent in Denmark.

### Table 1. Bacterial genuses/species isolated using routine aerobic cultivation.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Species</th>
<th>n</th>
<th>Species</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>37</td>
<td>Listeria monocytogenes</td>
<td>7</td>
<td></td>
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<tr>
<td>Acinetobacter spp.</td>
<td>15</td>
<td>Vibrio spp.</td>
<td>6</td>
<td></td>
<td></td>
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<tr>
<td>Achromobacter spp.</td>
<td>13</td>
<td>Vagococcus spp.</td>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td>Lactococcus spp.</td>
<td>9</td>
<td>Bacillus licheniformis</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>5</td>
<td>Lactobacillus spp.</td>
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<td></td>
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</tr>
<tr>
<td>Enterococcus spp.</td>
<td>8</td>
<td>Klebsiella spp.</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### SECOND GENERATION SEQUENCING

- **Neosporosis** was the most frequently diagnosed infection.
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### DISTRIBUTION OF ORGAN LESIONS

Figure 3. Placenta. Example of a cotyledonary villus with extended and necrotic trophoblasts, containing large numbers of intravillous bacterial colonies (green). Bacterial localisation and morphology will be used to choose relevant species-specific probes for further identification of intravillous bacteria.

Figure 4. Placenta. Multiple cotyledonary villi invaded by fungal septate and branching hyphae. Fungal structures have morphology will be used to choose relevant species-specific probes for further identification of intravillous bacteria.

Figure 5. DNA was extracted from placenta, a liver and lung pool as well as kidney. Part of the total bacterial DNA per sample was amplified using universal bacterial primers binding adjacent to the variable regions V1 and V2 of the 16S rRNA gene. The resulting amplicon was sequenced on the Illumina MiSeq platform.

Figure 6. Distribution of organ lesions diagnosed in HE tissue sections shown as number and percentage of abortions.

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