Evaluation of pre-PCR processing approaches for enumeration of Salmonella enterica in naturally contaminated animal feed

Lölström, Charlotta; Schelin, J.; Andersson, G.; Vigre, Håkan; Norling, B.; Häggblom, P.; Hoorfar, Jeffrey; Rådström, P.

Publication date: 2013

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Evaluation of pre-PCR processing approaches for enumeration of *Salmonella enterica* in naturally contaminated animal feed

C. Löfström\(^a\), J. Schelin\(^b\), G. Andersson\(^c\), H. Vigre\(^a\), B. Norling\(^d\), P. Häggblom\(^a\), J. Hoorfara\(^b\), & P. Rådström\(^b\)

\(^a\)National Food Institute, Technical University of Denmark, Søborg; \(^b\)Applied Microbiology, Lund University, Sweden; \(^c\)National Veterinary Institute (SVA), Uppsala, Sweden; \(^d\)Quintessence Research AB, Alunda, Sweden

**Aim**
To evaluate 3 pre-PCR processing strategies:
1. flotation-qPCR (modified from [1])
2. MPN-PCR (modified from [2])
3. qualitative culture enrichment PCR [2]
for the detection and/or quantification of *Salmonella* in naturally contaminated soy bean meal.

**Introduction**
Animal feed might serve as a reservoir of *Salmonella* contributing to the spread into the food chain. Levels of *Salmonella* in feed samples are low, bacteria are unevenly distributed and stressed and could therefore be hard to recover using standard culture-based methods. Due to this, there is a need for accurate, sensitive, rapid and user-friendly sample preparation methods prior to molecular analyses. Moreover, to facilitate quantitative risk assessment in the feed production chain, there is a need to enumerate *Salmonella* in naturally contaminated feed.

**Methods**

![Figure 1. Overview of the three pre-PCR processing strategies that were investigated. Bags of naturally contaminated soy bean meal (n = 15) were analyzed in parallel with the three methods.](Photo: By Roosewelt Pinheiro/Abr [CC-BY-3.0-br], via Wikimedia Commons)

<table>
<thead>
<tr>
<th>Method</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flotation-qPCR</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>MPN-PCR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Culture enrichment PCR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Conclusions**
- The 3 methods provide possibilities to assess the prevalence of *Salmonella* in feed, as well as the numbers of culturable, and non-culturable cells
- Differences in results could be due to non-culturable *Salmonella* and/or a heterogeneous distribution of *Salmonella* in the feed

**Results**
Out of the 15 bags analyzed 6, 15 and 9 were positive for *Salmonella* with flotation-qPCR, MPN-PCR and culture enrichment PCR, respectively (Table 1).

Enumeration resulted in values of $1.8 \times 10^2$-$7.8 \times 10^3$ CFU/g (flotation-qPCR) and 0.024 to >5.2 MPN/g (MPN-PCR) (Figure 2).

**Acknowledgements**
This study was financially supported by the EU project BIOTRACER (contract no. 036272) and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS).

**References**
1. Löfström et al. (2011) IJFM 145 Suppl 1:S103-109