Evaluation of the PrioCHECK® Trichinella AAD kit to detect *Trichinella britovi*, *T. spiralis* and *T. pseudospiralis* in muscle tissues of domestic pigs by the automated digestion method Trichomatic-35®

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Objective

- Evaluation of the PrioCHECK® Trichinella AAD kit in combination with the automated digestion method Trichomatic-35® for detection of larvae from encapsulated and non-encapsulated *Trichinella* species in pork samples
- Standard of comparison: Pepsin-HCl digestion using the Trichomatic-35® instrument

PrioCHECK® Trichinella AAD kit (Thermo Fisher Scientific)  Trichomatic-35® (Foss Germany, GmbH)  Pepsin-HCl (Opopharma Handels GmbH, Germany)
Materials and Methods

PrioCHECK® Trichinella AAD kit
(Thermo Fisher Scientific)

- Listed in European Commission (EC) Regulation 2015/1375 (Chapter II/E: “Artificial digestion test for in vitro detection of *Trichinella* spp. larvae in meat samples, PrioCHECK® Trichinella AAD Kit”) as an equivalent to the magnetic stirrer method using pepsin–HCl (reference method of detection) for testing meat of domestic swine
- In Switzerland it is also authorized (FSVO) for testing wild boar and horse meat since 1.12.2016
Materials and Methods

PrioCHECK Trichinella AAD Kit

3 Components:

1- Digestion **buffer** (20x)
2- Enzyme solution
3- Digestion buffer **additive**

- Protocol similar to the pepsin-HCl artificial digestion test
- Uses a **recombinant enzyme** (serin-endopeptidase) **instead of pepsin**
- Digestion at **60°C**
- **No HCl** used
- Digestion at a **slightly alkaline pH**
Materials and Methods

Trichromatic-35® blender with filtration insert + pepsin-HCl

- Listed in European Commission (EC) Regulation 2015/1375 (Chapter II/ C “Automated digestion method for pooled samples of up to 35 g”) as an equivalent to the reference method
- Transparent polycarbonate membrane filters with a diameter of 50 mm and a pore size of 14 µm
- **Pepsin**: 7g (strength 1:10,000 NF; 1:12,500 BP; 2,000 FIP)
- **HCl 8.5 ± 0.5%**: 30 ml
- Water: 400 ml
- Meat sample: **35 g**
Materials and Methods

Trichromatic-35® blender with filtration insert + AAD Kit

- Comp. 1 (Digestion buffer): 30 ml
- Comp. 2 (Enzyme): 30 ml
- Comp. 3 (Additive): 250 µl
- Water: 510 ml
- Meat sample: 35 g

30 ml in HCl chamber
380 ml in water chamber
160 ml in reaction chamber
## Materials and Methods

<table>
<thead>
<tr>
<th></th>
<th>Pepsin-HCl</th>
<th>AAD kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichromatic-35® instrument</td>
<td>standard</td>
<td>ad-hoc adapted*</td>
</tr>
<tr>
<td>Fast rotation time</td>
<td>3.5 min</td>
<td>2.5 min</td>
</tr>
<tr>
<td>Digestion temperature</td>
<td>49°C</td>
<td>60°C</td>
</tr>
<tr>
<td>Digestion time</td>
<td>8 min</td>
<td>8 min</td>
</tr>
</tbody>
</table>

*Moritz Geräte-reparatur UG (Rellingen, Germany)
Materials and Methods

*T. britovi*

Samples

10 pork samples (35g)

Digestion

- AAD Kit
  - Lot 1
- Pepsin-HCl

3 samples: 3 L
3 samples: 6 L
3 samples: 15 L
1 sample: 0 L

Total: 72 L
Materials and Methods

\textbf{T. spiralis}

**Samples**

10 pork samples (35g)

\begin{itemize}
  \item 3 samples: 3 L
  \item 3 samples: 6 L
  \item 3 samples: 15 L
  \item 1 sample: 0 L
\end{itemize}

**Digestion**

\begin{itemize}
  \item AAD Kit
    \begin{itemize}
      \item Lot 1
      \item Lot 2
      \item Lot 3
    \end{itemize}
  \item Pepsin-HCl
\end{itemize}

\textbf{Total: 72 L}

- In order to detect potential differences between batches of the AAD kit, the trial was repeated with 3 different production lots (1-3) on \textit{T. spiralis}-spiked samples.
Materials and Methods

*T. pseudospiralis*

Samples

- 16 pork samples (35g)

Digestion

- AAD Kit
  - Lot 1
- Pepsin-HCl

15 samples: 0.1 g muscle/sample

(unknown nr. of L)

1 sample: 0 L
## Results: *T. britovi*

| Spiked L | AAD Kit | | | Pepsin-HCl | |
|----------|---------|---------|---------|---------|
|          | detected | recovery (%) | damaged | detected | recovery (%) | damaged |
| 3        | 3        | 100      | -       | 3        | 100      | -       |
| 3        | 3        | 100      | -       | 3        | 100      | -       |
| 3        | 2        | 66.7     | -       | 3        | 100      | -       |
| 6        | 6        | 100      | -       | 6        | 100      | -       |
| 6        | 5        | 83.3     | 1       | 6        | 100      | -       |
| 6        | 5        | 83.3     | -       | 5        | 83.3     | -       |
| 15       | 12       | 80       | -       | 15       | 100      | -       |
| 15       | 11       | 73.3     | -       | 15       | 100      | 1       |
| 15       | 14       | 93.3     | 2       | 12       | 80       | -       |
| 0        | 0        | 0        | -       | 0        | 0        | -       |
| **100%** | **84.7 %** | **86.7%** | **3** | **94.4%** | **95.9%** | **1** |
| **n=72** | **n=61** | **(mean)** | | **(mean)** | | |

The differences are not statistically significant (*p* > 0.05)
**T. britovi** larvae recovered after **AAD** digestion

non-damaged *T. britovi* larvae

damaged *T. britovi* larvae

4.9\% (3/61)
*T. britovi* larvae recovered after **pepsin-HCl** digestion

non-damaged *T. britovi* larvae

damaged *T. britovi* larvae 1.5% (1/68)
## Results: *T. spiralis*

The differences among the AAD lots and respect to pepsin-HCl digestion are not statistically significant ($p>0.05$)
*T. spiralis* larvae recovered after AAD digestion

non-damaged *T. spiralis* larvae

damaged *T. spiralis* larvae

5.8% (11/191)
*T. spiralis* larvae recovered after *pepsin*-HCl digestion

non-damaged *T. spiralis* larvae

damaged *T. spiralis* larva

3% (2/66)
Recovered *T. spiralis* larvae removed from the filter

**AAD digestion**

**pepsin-HCl digestion**
### Results: *T. pseudospiralis*

<table>
<thead>
<tr>
<th>AAD Kit</th>
<th>Pepsin-HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>sample No.</strong></td>
<td><strong>detected L</strong></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td>263</td>
</tr>
<tr>
<td>9</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td>11</td>
<td>62</td>
</tr>
<tr>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>13</td>
<td>89</td>
</tr>
<tr>
<td>14</td>
<td>61</td>
</tr>
<tr>
<td>15</td>
<td>97</td>
</tr>
<tr>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,149</strong></td>
</tr>
<tr>
<td>Mean</td>
<td>76.6</td>
</tr>
</tbody>
</table>

95% CI [48.5, 104.7] 95% CI [58.7, 108.4]

The difference is not statistically significant at $p < 0.05$ ($p = 0.3601$)
Recovered *T. pseudospiralis* larvae

non-damaged larvae after **pepsin-HCl** digestion

non-damaged larvae after **AAD** digestion
Recovered *T. pseudospiralis* larvae

damaged *T. pseudospiralis* larvae after **AAD** digestion
## Results: Total recovery rates

<table>
<thead>
<tr>
<th></th>
<th>Total L recovery rate (n=72 L)</th>
<th>Detected L/sample (mean) [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T. britovi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAD Kit 1</td>
<td>84.7% (61 L)</td>
<td>86.7% [78.6, 94.7]</td>
</tr>
<tr>
<td>Pepsin-HCl</td>
<td>94.4% (68 L)</td>
<td>95.9% [90.6, 100]</td>
</tr>
<tr>
<td><strong>T. spiralis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAD Kit 1</td>
<td>80.5% (58 L)</td>
<td>83.3% [67.9, 98.7]</td>
</tr>
<tr>
<td>AAD Kit 2</td>
<td>88.9% (64 L)</td>
<td>87.8% [78.5, 97.0]</td>
</tr>
<tr>
<td>AAD Kit 3</td>
<td>95.8% (69 L)</td>
<td>93.7% [86.1, 100]</td>
</tr>
<tr>
<td>Pepsin-HCl</td>
<td>91.7% (66 L)</td>
<td>86.4% [71.4, 100]</td>
</tr>
<tr>
<td><strong>T. pseudospiralis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAD Kit 1</td>
<td>1,149</td>
<td>76.6 [48.5, 104.7]</td>
</tr>
<tr>
<td>Pepsin-HCl</td>
<td>1,253</td>
<td>83.5 [58.7, 108.4]</td>
</tr>
</tbody>
</table>
Conclusions

• The PrioCHECK Trichinella AAD kit in combination with an adapted Trichromatic-35® device was easy to use and achieved a good digestion of pork samples (undigested material <0.1 g in all cases)

• All positive (n=84) and negative (n=8) samples were correctly identified in all trials by both methods independent of the Trichinella species and AAD kit lot

• Larvae recovery rates were comparable to the standard artificial digestion method based on pepsin and HCl

• The quantitative differences were not statistically significant

• Therefore, it seems to fulfill the requirements as an alternative method for the detection of Trichinella spp. in pig meat
Conclusions

• However, the morphological structure of the recovered larvae appeared to be more frequently affected after digestion with the AAD kit (especially for *T. pseudospiralis*).

• In some individual larvae, the visualization of the internal structure characteristics for the genus *Trichinella* such as the stichosome was not possible.

• In case that only damaged larvae would be present in a sample, a PCR-based identification of such larvae could be a solution to this hint.

• Federal Food Safety and Veterinary Office (FSVO) is currently deciding on the authorization of this test in Switzerland.
Aknowledgements

• Thomas Moritz
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  and Veterinary Office

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra