Relationship between seroprevalence in the main livestock species and presence of Toxoplasma gondii in meat

GP/EFSA/BIOHAZ/2013/01
Project Results 2013-2015

Relationship between seroprevalence in the main livestock species and presence of Toxoplasma gondii in meat


Objectives

- To carry out an extensive literature review of available data on *T. gondii* in meat of the main livestock species.
- To perform experimental studies on *T. gondii* in meat-producing livestock species in the EU.

**Main Livestock species:**
- Cattle
- Sheep & Goat
- Horses
- Pigs
- Chicken
- Turkey

**Objective 1**

To carry out an extensive literature search and review of available data on *T. gondii* in meat of the main livestock species.

1. The anatomical distribution of the cysts in meat and other edible tissues, to inform the optimal sampling choice(s) for slaughtered animals for optimisation of detection (WP2)
2. Available methods for detecting the presence and infectivity of *T. gondii* cysts, including their sensitivity and specificity (WP2)
3. Relationship between seroprevalence in the main livestock species and presence and infectivity of *T. gondii* cysts in their meat and other edible tissues (WP2)
4. Risk factors for *T. gondii* infection in the main livestock species: (WP3)

**Objective 2**

To perform experimental studies on *T. gondii* in meat-producing livestock species in the EU based on data gaps in the literature.

1. Relationship indirect detection methods (e.g. *T. gondii* seroprevalence) and direct detection methods for presence and levels of infective cysts in meat and other edible tissues
2. Anatomical distribution of the cysts in meat and other edible tissues, to inform the optimal sampling choice(s) for slaughtered animals
3. Identify on-farm risk factors for *T. gondii* infection in each animal species
### Extensive literature search: systematic review approach

- 1766 records
- 115 Q
- 537 Title
- 362 Abstract
- 317 Full-text
- 18,777
- 267 WP2
- 75 WP3

### Anatomical distribution

<table>
<thead>
<tr>
<th>Species</th>
<th>Top 5 tissues</th>
<th>Summed score (W)</th>
<th>Number of records (studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td>brain, heart, tongue, diaphragm, choroid-retinal coat</td>
<td>1.44-0.79</td>
<td>30 (37)</td>
</tr>
<tr>
<td>Cattle</td>
<td>skirt steak, unspecified lymph nodes, thigh muscle, small intestine, liver</td>
<td>0.73-0.62</td>
<td>10 (19)</td>
</tr>
<tr>
<td>Sheep</td>
<td>brain, heart, skeletal muscle, thorax muscles/flanks, diaphragm</td>
<td>1.30-0.77</td>
<td>12 (17)</td>
</tr>
<tr>
<td>Goats</td>
<td>kidneys, brain, heart, liver, skeletal muscle</td>
<td>1.51-1.41</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Chickens</td>
<td>heart, brain, ovary duct, ovaries, ventriculus</td>
<td>1.47-0.80</td>
<td>19 (21)</td>
</tr>
<tr>
<td>Turkeys</td>
<td>heart, brain, limb muscle, liver, thigh muscle</td>
<td>1.50-1.09</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Horses</td>
<td>heart, tongue, small intestine, brain, spinal cord</td>
<td>1.53-0.99</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>

- Limited data for turkeys and horses
- Predilection site varies by species, brain and heart in top 5 for pigs, sheep, goats, chickens, turkeys and horses
- Cattle little overlap with other species and low scores

### Selection of tissues for experimental studies

- **Cattle (WP5)**: Liver and heart
- **Pigs (WP6)**: Heart and diaphragm
- **Horses (WP7)**: Heart and diaphragm
- **Chickens (WP8)**: Heart and drumstick and lower leg muscle

### Introduction of direct detection

- Tissue cysts
- 1 per 50g
- Hundreds to thousand of bradyzoites
Direct detection methods

Frequency of direct detection methods in 502 entries of results (from 281 publications)

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse bioassay</td>
<td>206</td>
</tr>
<tr>
<td>PCR</td>
<td>124</td>
</tr>
<tr>
<td>Microscopy without specific staining</td>
<td>59</td>
</tr>
<tr>
<td>Cat bioassay</td>
<td>52</td>
</tr>
<tr>
<td>IHC or IFAT</td>
<td>24</td>
</tr>
<tr>
<td>Antigen-ELISA (antibody-based detection of circulating antigens)</td>
<td>13 (4 publications)</td>
</tr>
<tr>
<td>LAMP</td>
<td>6 (4 publications)</td>
</tr>
<tr>
<td>In vitro isolation</td>
<td>3 (1 publication)</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
</tr>
</tbody>
</table>

Conclusions performance direct detection methods

- 1: Cat bioassay
- 2: Mouse bioassay
- PCR can perform similarly to mouse bioassay depending on sampling and protocol details.
- Limited information available for LAMP, in vitro methods and detection of circulating antigens
- Microscopy-based methods lack sensitivity
- Variation in the ranking is influenced by the total volume tested (sample size and number of samples)

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Detection of antibodies and presence of tissue cysts

- Lifelong persistence of antibodies and tissue cysts
- Theoretically, only lack of correlation when:
  - Recent infection: antibody response not yet detectable
  - Tissue cysts are sparse, can be missed when sampling for direct detection
- Other potential issues, for example: Autolysis of samples, PCR contamination, false identification by microscopy, misclassification by serological method
- If the correlation is strong: serological methods (cheaper and easier for large-scale screening) can be used to get an indication of the presence of tissue cysts.

Results

<table>
<thead>
<tr>
<th>Species</th>
<th>Detection in seropositives</th>
<th>Detection in seronegatives</th>
<th>Entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipis</td>
<td>Overall 58.8% (n=592)</td>
<td>Range 8-100%</td>
<td>Overall 0-75%</td>
</tr>
<tr>
<td>Cattle</td>
<td>3.4% (n=111)</td>
<td>0-10%</td>
<td>2.4% (n=457)</td>
</tr>
<tr>
<td>Sheep</td>
<td>39.4% (n=1002)</td>
<td>5-100%</td>
<td>1.8% (n=922)</td>
</tr>
<tr>
<td>Goats</td>
<td>34.9% (n=152)</td>
<td>0-72%</td>
<td>2.3% (n=50)</td>
</tr>
<tr>
<td>Chickens</td>
<td>53.4% (n=1679)</td>
<td>0-100%</td>
<td>1.8%-17.4% (n=2133)*</td>
</tr>
<tr>
<td>Horses</td>
<td>&lt;8.8%-13.8% (n=86)</td>
<td>8-9%</td>
<td>2.4%-32.0% (n=549)*</td>
</tr>
</tbody>
</table>

*Ranges (chickens and horses) due to pooled sampling
**Risk factors**

Available studies per species by continent

For cattle, equids, and poultry there were almost no studies available.

<table>
<thead>
<tr>
<th>Continent</th>
<th>Cattle</th>
<th>Chickens</th>
<th>Goats</th>
<th>Equids</th>
<th>Pigs</th>
<th>Sheep</th>
<th>Sheep and goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Asia</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Europe</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>27</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>North America</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td>1</td>
<td>20</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>South America</td>
<td>10</td>
<td>6</td>
<td>47</td>
<td>25</td>
<td>10</td>
<td>1</td>
<td>111</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>9</td>
<td>4</td>
<td>10</td>
<td>6</td>
<td>47</td>
<td>25</td>
<td>111</td>
</tr>
</tbody>
</table>

**Conclusions risk factors**

- Conclusions for factors considered biologically relevant:
  - The presence of cats or detection of *T. gondii* oocysts were always identified as risk factors (pigs, small ruminants).
  - Likely fodder contamination increased risk (pigs, goats).
  - Low level of confinement was associated with increased risk (pigs, chickens), but also with protection in a few studies (pigs, cattle).
  - Variables suggesting a likely transmission via rodents were associated with risk (pigs, sheep). However, when variables suggested unlikely transmission via rodents, this revealed either risk or protection (pigs, sheep).
  - Variables characterizing contamination of drinking water or level of management intensity revealed no clear effect.
- Further studies are necessary to solve conflicting findings and to complete knowledge. There is a need for experimental studies to confirm the validity of findings of cross-sectional studies.

**Objective 2**

To perform experimental studies on *T. gondii* in meat-producing livestock species in the EU based on data gaps in the literature.

1. Relationship indirect detection methods (e.g. *T. gondii* seroprevalence) and direct detection methods for presence and levels of infective cysts in meat and other edible tissues
2. Anatomical distribution of the cysts in meat and other edible tissues, to inform the optimal sampling choice(s) for slaughtered animals
3. Identify on-farm risk factors for *T. gondii* infection in each animal species

**Experimental Studies (10x)**

- **Ruminants**
  - Cattle slaughterhouse
  - Experimental infection in calves
  - Vaccination and challenge in sheep
  - Risk factors in indoor-housed dairy goats
- **Pigs**
  - Slaughterhouse study France
  - Vaccination and challenge
  - Risk factors UK
- **Horses**
  - Slaughterhouse study
  - Poultry
  - Farm study organic laying hens
  - Experimental infection chickens and turkeys
Cattle study in 4 countries sampling

- 4 countries (UK, IT, RO, NL), 100 cattle each
- Adult cattle and calves 50:50, except in UK (84:16)
- Serum for MAT (in France): all cattle
- Liver for mouse bioassay and qPCR on digest: all cattle
- Diaphragm for MC-PCR (in NL): 100 mouse bioassay and digest-PCR negative cattle (irrespective of serological result) plus cattle positive in mouse bioassay, digest-PCR or by MAT

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th>Calf</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>191</td>
<td>151</td>
<td>342</td>
</tr>
<tr>
<td>positive</td>
<td>44 (18.7%)</td>
<td>16 (9.6%)</td>
<td>60 (14.9%)</td>
</tr>
</tbody>
</table>

Pearson's χ² p-value=0.011

Results direct detection

<table>
<thead>
<tr>
<th></th>
<th>UK</th>
<th>IT</th>
<th>RO</th>
<th>NL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse bioassay</td>
<td>0/85</td>
<td>4/100</td>
<td>0/100</td>
<td>2/100</td>
<td>6/385</td>
</tr>
<tr>
<td>serology mice</td>
<td>0/84</td>
<td>0/100</td>
<td>0/55</td>
<td>0/100</td>
<td>0/179</td>
</tr>
<tr>
<td>PCR mouse brain</td>
<td>0/85</td>
<td>4/100</td>
<td>0/100</td>
<td>2/100</td>
<td>6/385</td>
</tr>
<tr>
<td>PCR digest</td>
<td>0/100</td>
<td>0/100</td>
<td>0/100</td>
<td>0/100</td>
<td>0/400</td>
</tr>
<tr>
<td>MC-PCR</td>
<td>0/34</td>
<td>0/30</td>
<td>7/44</td>
<td>0/43</td>
<td>7/151</td>
</tr>
</tbody>
</table>

- 13 out of 402, 6 by mouse bioassay
- No overlap in positive results from the different methods

Concordance direct and indirect detection

<table>
<thead>
<tr>
<th></th>
<th>MBio</th>
<th>PCR digest</th>
<th>MC-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT &lt;1:6</td>
<td>4/325 (1.2%)</td>
<td>0/341 (0.0%)</td>
<td>4/92 (4.3%)</td>
</tr>
<tr>
<td>MAT ≥1:6</td>
<td>2/60 (3.3%)</td>
<td>0/60 (0.0%)</td>
<td>3/59 (5.1%)</td>
</tr>
<tr>
<td>kappa</td>
<td>0.033</td>
<td>0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Difference in recovery by direct detection in seronegatives and positives is not statistically significant for any of the methods (Fisher’s exact test).

Conclusions

- T. gondii has been detected in 13 out of 402 cattle and in 6 of these detection was based on mouse bioassay (1.6%), indicating the presence of viable parasites.
- The data are not suitable to compare the parasite load in liver and diaphragm or the performance of mouse bioassay and MC-PCR, but the number of parasites appears to be low in bovine liver and diaphragm.
- MAT appears unsuitable to obtain an estimate of the prevalence of viable T. gondii in cattle and does not provide an indication of the risk for consumers.
- The discordance is present whichever direct detection method is considered, and could not be resolved by the use of other serological assays. For that reason, the lack of concordance between methods likely represents a true lack of correlation between the presence of antibodies and the presence of (viable) T. gondii in cattle.
Experimental infection in calves (Moredun)

- 6 Holstein-Friesian calves
- orally infected with $1 \times 10^6$ T. gondii oocysts of the M4 strain
- Six weeks
- IgG antibody detection by MAT
- Tissues tested in 2 pools by mouse bioassay, qPCR on digest and MC-PCR
- All six seroconverted
- One calf died to unrelated disease before the end of the trial

Conclusions

- After oral inoculation with $10^2$ oocysts of calves, both viable T. gondii and DNA were detected in various tissues including meat cuts.
- Semitendinosus and tongue gave consistent mouse bioassay results and were among the samples with lower Cq-values in MC-PCR, but many other tissues of pool 2 also tested positive in both methods, therefore no clear predilection sites were identified.

Vaccination and challenge in sheep (Moredun)

- MC-PCR in addition to previously published data (Katzer et al., 2014)
- Vaccination with $1.2 \times 10^5$ S48 tachyzoites 4 weeks before infection then infection with $5 \times 10^5$ M4 T. gondii oocysts, culled at 28 or at 42 days post infection.
- Infection with $5 \times 10^5$ M4 T. gondii oocysts, culled at 28 or at 42 days post infection.
Vaccination and challenge in sheep

- T. gondii DNA was present at similar concentrations in various edible tissues of oocyst and tissue cyst inoculated lambs.
- Vaccination using S48-strain tachyzoites reduces parasite load in edible tissues of lambs.
- No MC-PCR based detection of tissue cysts in vaccinated sheep.
- Analysis of a broader range of tissues did detect T. gondii DNA by ITS1 PCR from distal jejuna/prescapular lymph nodes, liver, kidney and skeletal muscle from lambs euthanised on 28 and 42 days p.i. (Katzer et al., 2014)

Risk factors indoor-housed Dutch dairy goats

- 52 farms, 32 goats per farm
- IDvet ELISA
- Questionnaire
- 13.3% goats (95% CI: 11.7%-14.9%)
- 61.5% farms (95% CI: 48.3%-74.7%)
- Number of cats

Slaughterhouse study pigs in France

- 160 out of 1549 heart samples (69 samples from pigs with MAT titre >1:6 and 91 samples from randomly selected seronegative pigs).
- Heart: mice bioassay and qPCR
- Diaphragms: MC-PCR
- MAT on cardiac fluid used as reference

Note: Only seropositive mice tested by PCR, and different qPCR protocol for digest

Concordance direct and indirect detection

- The antibody detection and the presence of parasites are positively correlated.
- The overall direct detection rate in antibody detection positive animals (80.4%) is significantly higher than the detection rate in antibody detection negative animals.
- Relatively high detection rate of T. gondii in seronegatives (34.9%) needs further investigation
Vaccination and challenge in pigs (Moredun)

- MC-qPCR testing of edible tissues in addition to published mouse bioassay and ITS1 PCR results (Burrells et al., 2015)
- Infection with 1000 M4 tissue cysts, culled 6 weeks post infection
- Infections with 1000 M4 oocysts, culled 6 weeks post infection
- Vaccination with \(1.2 \times 10^5\) S48 tachyzoites 4 weeks before infection with 1000 sporulated M4 T. gondii oocysts.

- 7. gondii was detected in various edible tissues in tissue cyst or oocyst inoculated animals, with little variation in parasite load
- 7. gondii was not detected in the vaccinated and challenged group, therefore the vaccine does not appear to induce tissue cysts and reduces tissues cyst burden in challenged animals.

Slaughterhouse study in horses

- France and Serbia, 100 horses collected (180 FR and 104 RS)
- Heart: mouse bioassay and qPCR on digest
- Diaphragm: MC-PCR (in France)
- Serum tested by MAT, cut-off \(\geq 1:6\)

<table>
<thead>
<tr>
<th>MAT</th>
<th>France</th>
<th>Serbia</th>
<th>Total</th>
<th>Pearson's (\chi^2) p-value=0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>128</td>
<td>54</td>
<td>182</td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>50 (27.8%)</td>
<td>50 (48.1%)</td>
<td>100 (55.5%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MAT</th>
<th>(&lt;2) yrs</th>
<th>(\geq2) yrs</th>
<th>Total</th>
<th>Pearson's (\chi^2) p-value=0.195</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>144</td>
<td>39</td>
<td>183</td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>85 (37.3%)</td>
<td>15 (27.8%)</td>
<td>100 (35.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Direct detection in horses in FR and RS

<table>
<thead>
<tr>
<th>Method</th>
<th>France</th>
<th>Serbia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse bioassay</td>
<td>2/175</td>
<td>56/133</td>
<td>58/278</td>
</tr>
<tr>
<td>PCR mouse brain</td>
<td>2/175</td>
<td>48/133</td>
<td>50/278</td>
</tr>
<tr>
<td>Microscopy on mouse brain</td>
<td>ND</td>
<td>10/133</td>
<td>10/133</td>
</tr>
<tr>
<td>Serology mice</td>
<td>0/175</td>
<td>2/103</td>
<td>2/278</td>
</tr>
<tr>
<td>PCR heart digest</td>
<td>1/175</td>
<td>28/134</td>
<td>29/279</td>
</tr>
<tr>
<td>MC-PCR diaphragm</td>
<td>12/180</td>
<td>8/104</td>
<td>20/284</td>
</tr>
<tr>
<td>Any direct detection method</td>
<td>15/180</td>
<td>68/134</td>
<td>83/214</td>
</tr>
</tbody>
</table>
Concordance direct and indirect detection

- Only microscopic examination was taken into account for mouse bioassay results from Serbia
- PCR of digest was excluded from these analyses

<table>
<thead>
<tr>
<th></th>
<th>MBio</th>
<th>MC-PCR</th>
<th>Any DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT &lt;1:6</td>
<td>4/178 (2.2%)</td>
<td>12/182 (6.7%)</td>
<td>16/184 (8.7%)</td>
</tr>
<tr>
<td>MAT ≥1:6</td>
<td>8/98 (8.2%)</td>
<td>8/100 (8.0%)</td>
<td>16/98 (16.3%)</td>
</tr>
<tr>
<td>kappa</td>
<td>0.08</td>
<td>0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>95%CI</td>
<td>-0.07-0.23</td>
<td>-0.13-0.17</td>
<td>-0.05-0.23</td>
</tr>
</tbody>
</table>

Conclusions horses

- 11.6% of horses were considered positive by direct detection, 4.2% by mouse bioassay, indicating the presence of viable tissue cysts.
- There was a lack of concordance between MAT and direct detection of *T. gondii*.
- No concordance between different direct detection methods, most likely reflecting a low concentration of *T. gondii* in positive horses.
- Significant difference between the qPCR based results in France and Serbia. Only the microscopical results were included in the final analyses.
- Since there was a significant difference in seroprevalence, further studies are needed in Serbia to assess the potential risk.
- Two strains of *T. gondii* have been isolated from horses in Serbia, which demonstrates the presence of viable *T. gondii* in horses.

Studies in poultry in Germany

1. Farm study in organic laying hens

- 1-3 year old laying hens from organic farms and backyard systems
- 470 tested serologically, 61 hens selected for direct detection methods
- Heart and drumstick both for mouse bioassay and MC-PCR
- Serum: Tg-SAG1 ELISA, IFAT and MAT
- Risk factors: Questionnaire

Note: Pepsin-digestion and use of gamma-interferon-knockout mice (GKO mice) or gamma-interferon-receptor-knockout mice (GRKO mice) and different PCR protocol for digests

Concordance direct detection methods

- Substantial concordance between direct detection methods
- Heart is more frequently positive than drumstick
Concordance direct and indirect detection

- Substantial concordance between direct and indirect detection

<table>
<thead>
<tr>
<th>Method</th>
<th>MBio</th>
<th>MC-PCR</th>
<th>Digest PCR</th>
<th>Any DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT</td>
<td>0.55</td>
<td>0.68</td>
<td>0.64</td>
<td>0.65</td>
</tr>
<tr>
<td>IFAT</td>
<td>0.64</td>
<td>0.67</td>
<td>0.71</td>
<td>0.70</td>
</tr>
<tr>
<td>ELISA (TgSAG1)</td>
<td>0.61</td>
<td>0.77</td>
<td>0.67</td>
<td>0.74</td>
</tr>
<tr>
<td>MajRef</td>
<td>0.80</td>
<td>0.90</td>
<td>0.87</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Kappa-values with 95%CI

Risk factors

- No bioassay positive hen was identified on large organic farms.

- In univariate analysis, variables related to the presence of domestic cats on farm, the use of cats for rodent control, a low chicken density on the chicken run and other variables linked to the small size of the farm were associated with a >20% seroprevalence (ELISA) on the farm.

Studies in poultry

2. Anatomical distribution in laying hens and turkeys

Experimental infections in laying hens and turkeys

Different strains and inoculation routes were used

Anatomical distribution in chickens and turkeys

- Chickens: heart and brain significant higher load than other tissues
- Turkeys: brain higher than heart, both higher than other tissues
General conclusions

- Relationship between direct and indirect detection
- Anatomical distribution of tissue cysts
- Risk factors

Relationship between indirect and direct detection of *T. gondii*

- The detection of antibodies appears to be useful to estimate the extent of viable *T. gondii* in pigs and poultry. However, viable *T. gondii* was also detected in <10% of seronegative pigs and chickens, therefore serological screening can not be used to declare the meat of individual animals as *T. gondii*-free.

- MAT-based detection of antibodies (possibly serological screening in general) is not recommended as an indicator of the presence of viable *T. gondii* in cattle and horses.

- In cattle and horses direct detection methods are preferred.

Anatomical distribution of *T. gondii* tissue cysts

Selection of tissues for the slaughterhouse studies:

- Cattle: No clear predilection sites were identified in the calf infection experiment, the selected tissues gave positive results in the experimental infection study.
- Pigs: Results from literature confirm that heart is a predilection site and the best choice for sampling. Diaphragm is still considered a suitable representative of edible tissue.
- Horses: The anatomical distribution of tissue cysts in horses remains an important gap in knowledge.
- Chickens: Brain and heart predilection sites

Direct detection in seropositives and seronegatives

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Detection in seropositive animals (total number examined)</th>
<th>Detection in seronegative animals (total number examined)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Mouse brain</td>
<td>1.3% (n=50)</td>
<td>1.5% (n=33)</td>
</tr>
<tr>
<td></td>
<td>qPCR digest</td>
<td>0.0% (n=60)</td>
<td>0.0% (n=34)</td>
</tr>
<tr>
<td></td>
<td>MC-PCR</td>
<td>0.3% (n=95)</td>
<td>1.8% (n=42)</td>
</tr>
<tr>
<td>Pigs</td>
<td>Mouse brain</td>
<td>90.5% (n=89)</td>
<td>6.0% (n=83)</td>
</tr>
<tr>
<td></td>
<td>qPCR digest</td>
<td>71.1% (n=82)</td>
<td>23.5% (n=88)</td>
</tr>
<tr>
<td></td>
<td>MC-PCR</td>
<td>30.8% (n=82)</td>
<td>10.2% (n=88)</td>
</tr>
<tr>
<td>Horses</td>
<td>Mouse brain</td>
<td>8.1% (n=99)</td>
<td>2.2% (n=78)</td>
</tr>
<tr>
<td></td>
<td>qPCR digest</td>
<td>0.0% (n=50)</td>
<td>0.0% (n=123)</td>
</tr>
<tr>
<td></td>
<td>MC-PCR</td>
<td>8.4% (n=150)</td>
<td>6.0% (n=182)</td>
</tr>
<tr>
<td>Organic hens</td>
<td>Mouse brain</td>
<td>95.7% (n=28)</td>
<td>6.1% (n=35)</td>
</tr>
<tr>
<td></td>
<td>qPCR digest</td>
<td>96.4% (n=28)</td>
<td>9.1% (n=33)</td>
</tr>
<tr>
<td></td>
<td>MC-PCR</td>
<td>96.4% (n=28)</td>
<td>6.1% (n=33)</td>
</tr>
</tbody>
</table>
Risk factors

- Cats are considered main risk factor studies in goats and poultry.
- Indoor-housed dairy goats seroprevalence appears to be relatively low. The presence of cats was associated with increased on-farm seroprevalence.
- For laying hens with outdoor access in Germany, the risk of T. gondii infection was higher in backyard systems or at small farms compared to large farms.
- Experimentally, vaccination with S48-strain T. gondii has been shown to reduce or prevent tissue cyst development in sheep and pigs.

Recommendations

Relationship between indirect and direct detection

- For pigs, poultry and small ruminants serological screening can be used to identify high risk herds or animals. However, a negative result in an indirect test can not be used to guarantee that the meat is safe.
- With currently available serological methods, implementation of serological screening to identify high risk herds or animals is not considered useful for cattle and horses.
- In sheep vaccination status should be considered if serological testing would be implemented to identify high risk herds or animals.

Recommendations

Anatomical distribution

- Further experimental studies for the purpose of studying the anatomical distribution in cattle are not advised unless more sensitive methods will become available.
- Experimental infection in horses is needed to study anatomical distribution and identify preferred tissues for testing.
- Direct testing of a predilection site (brain and heart) in pigs, small ruminants and poultry will make most efficient use of resources to determine the prevalence of animals harbouring tissue cysts.

Recommendations

Risk factors

- Risk factors in cattle and horses studies should be based on a direct rather than on currently available indirect detection methods.
- Housing information is unlikely to be useful for risk classification in indoor kept goats.
- The possibility to reduce T. gondii transmission to consumers by vaccination of sheep and pigs should be further evaluated.
- Intervention studies are needed to determine the effectiveness of preventing exposure to risk factors.
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Two EFSA EXTERNAL SCIENTIFIC REPORT reports:
