Epidemiology, diagnosis and control of *Toxoplasma gondii* in animals and food stuff

Aize Kijlstra
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Toxoplasmosis is a neglected disease entity

- Disease burden is similar to salmonellosis and campylobacteriosis
- Treatment results are disappointing
- No human vaccines available
- More emphasis is needed on prevention
- A *Toxoplasma* safe meat chain is a feasible preventive approach
Outline of the talk

- Meat as a source of *Toxoplasma* infection
- Diagnostic methods
- Most important food animals
- Meat and meat products
- Control and politics
Meat as a source of human *Toxoplasma* infection

- Toxoplasmosis outbreaks associated with raw meat consumption
- Epidemiological studies in humans
  - 30-63% cases attributable to meat (Cook et al 2000)
- *T. gondii* infection lower in strict vegetarians
- Decline human *T. gondii* infection parallels decreased seroprevalence in animals
Consumption of meat products is the main cause of toxoplasmosis in Europe

Sheep, pigs, goats +++
poultry, deer, rabbit ++
Horse +
Cattle -

Tenter et al 2000
Other food stuff and water as a source of *Toxoplasma* infection

- **Water**
  - Infection via oocysts from cat feces
  - Important source in underdeveloped countries

- **Vegetables**
  - Oocyst contamination (never proven)

- **Shellfish**
  - Filtration of water contaminated with oocysts
  - Role in infection of marine mammals

- **Milk and milk products**
  - Exact role not yet clear

- **Eggs**
  - Unlikely
Diagnostic methods: parasite detection

- Histology immunohistochemistry
- In vitro culture
- Bioassay (golden standard)
  - Mouse inoculation; 3-4 wks; serology; examine brains
  - Cat feeding; examine oocyst secretion feces
- PCR

“cyst density is low; enrichment needed”
## Diagnostic methods: serology

- Modified agglutination test (MAT)
- Immunofluorescence antibody test (IFAT)
- ELISA
- Latex agglutination test

“All edible parts of a seropositive pig should be considered infectious”
Diagnostic methods: problems

- No standardisation
  - Different protocols
  - Different *Toxoplasma* strains
  - Different cut off points
- Reference materials not available
- Cross reactions with other parasites
## Animal Toxoplasma seroprevalence in Europe

<table>
<thead>
<tr>
<th>Meat animal</th>
<th>Seroprevalence with range</th>
<th>Isolation of viable parasite from meat cuts from naturally infected animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Game</td>
<td>56% (0-100%)</td>
<td>yes</td>
</tr>
<tr>
<td>Sheep</td>
<td>36% (4-92%)</td>
<td>yes</td>
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<tr>
<td>Cow</td>
<td>34% (2%-92%)</td>
<td>no</td>
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<tr>
<td>Goat</td>
<td>33% (0-77%)</td>
<td>yes</td>
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<tr>
<td>Horse</td>
<td>26% (0-80%)</td>
<td>yes</td>
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<tr>
<td>Chicken</td>
<td>10% (5-36%)</td>
<td>yes</td>
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<tr>
<td>Pig</td>
<td>6% (0-64%)</td>
<td>yes</td>
</tr>
</tbody>
</table>

Kijlstra and Jongert, Trends Parasitol. 2009
Dramatic decrease of Toxoplasma infection in Dutch Slaughter Pigs
Public demand for improved animal welfare

Regular ("bio-industry"):  
- Pigs always inside  
- No bedding materials  
- Conventional feed  
- Welfare low

Organic or Free range:  
- Pigs allowed outside  
- Roughage/ straw bedding  
- No tail or teeth clipping  
- Improved welfare
Effect of farm type on toxoplasma infection of slaughter pigs (Netherlands 2001)

One pig is consumed by 200-400 individuals.
Frequency of toxoplasma seropositive pigs on 41 organic pig farms (2796 pigs in total) (year: 2004)
Case report: pig farm with 93% toxoplasma seropositive pigs

- Farmer always fed goat whey to his pigs

- Goat milk as cause of toxoplasma infection
Non-pasteurised goat milk products as a cause of toxoplasmosis
Controlling Toxoplasma presence in meat

- Pre-harvest measures
- Post-harvest measures
Risk Factors involved in toxoplasma infection in Animal Production systems

- Outdoor access
  - (cat feces, drinking surface water)
- Presence of Cats
- Rodent control
- Feeding roughage/straw bedding
  - (cat feces, rodents)
- Feeding Goat Whey
# Pre-harvest measures for prevention of *T. gondii* infection

<table>
<thead>
<tr>
<th>Risk</th>
<th>Prevention measure</th>
<th>Measure outcome</th>
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<tbody>
<tr>
<td>Active oocyst shedding</td>
<td>Remove cat from farm</td>
<td>No oocyst source on farm</td>
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<tr>
<td></td>
<td>Vaccinate cat</td>
<td>Reduce or prevent oocyst shedding</td>
</tr>
<tr>
<td>Environmental oocyst contamination</td>
<td>Sterilize feed and bedding, no outdoor access</td>
<td>Reduction of exposure to oocysts</td>
</tr>
<tr>
<td><em>Toxoplasma</em> infected rodents</td>
<td>Rodent control program</td>
<td>Reduction of transmission of <em>Toxoplasma</em> to carnivorous meat animals</td>
</tr>
<tr>
<td>Tissue cysts in meat</td>
<td>Vaccinate farm animals</td>
<td>Prevent tissue cyst formation</td>
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<tr>
<td>Prevention program</td>
<td>Inform people at risk</td>
<td>Awareness of exposure risks</td>
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</table>
The Netherlands has 16 million inhabitants keeping 3.4 million cats.
Percentage of pig farm types using active rodent control with rodenticides

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<tr>
<th>Type</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Regular</td>
<td>100%</td>
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<tr>
<td>Free-range</td>
<td>86%</td>
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<tr>
<td>Organic</td>
<td>69%</td>
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</table>
Effect of rodent control on *Toxoplasma* seroprevalence in pigs
Monitoring animals at slaughter

- no general agreement among the tests to be used
- no standardized reference sera or other reference materials are available
- no laboratory certification program.
Toxoplasma in grazing animals
(sheep, goats, horses)

- As long as environmental contamination remains high, no change in Toxoplasma status expected
- Risk groups in population handling & eating sheep meat (French; moslims)
“Farm to fork” prevention of Toxoplasmosis

- **Pre Harvest**
  - Farm management (cats, rodent control, feed)
  - Vaccination
  - Slaughter house toxoplasma monitoring

- **Post Harvest**
  - Alternative processing of meat from infected animals (freezing)
  - Retailer (cooking instructions on package)
  - Consumer education
Killing of *T. gondii* tissue cysts in meat

- heat treatment
- freezing
- irradiation
- high pressure
- acidity and enhancing solutions
Toxoplasma gondii inactivation by heat treatment of pork meat
## Effect of salt on T. gondii survival

<table>
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<tr>
<th>NaCl concentration (%)</th>
<th>Temperature °C</th>
<th>Days</th>
<th>0.85</th>
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<th>3.3</th>
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Conclusions post harvest meat treatment

- Methods used by butchers to make sausages and ham products of organic pig meat are frequently not sufficient to kill the *Toxoplasma gondii* parasite.
- Only few processors freezes the meat in advance, and as a result the parasite is made harmless.
- Most processors, when preparing the meat products use salt concentrations, which according to literature, are not sufficient to kill the parasite within a reasonable time period.
### Post-harvest measures for killing *T. gondii* cysts in meat

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<th>Prevention measure</th>
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<td>Monitoring programme at slaughter</td>
<td><em>Toxoplasma</em> positive meat selected for pre-heated or frozen meat products.</td>
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<td></td>
<td>Grant a <em>Toxoplasma</em> free meat label or status to product and farm.</td>
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<tr>
<td>Cook meat well at &gt; 56°C for 10 minutes</td>
<td>Tissue cysts are killed.</td>
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<td>Sell cured meat with &gt;2% salt after three-four weeks storage</td>
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<tr>
<td>Freeze meat for at least 2 days at temperatures &lt; -12°C</td>
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<tr>
<td>Improved kitchen hygiene</td>
<td>Reduction of cross-contamination</td>
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How to obtain a *Toxoplasma* safe meat chain

- Validated tests are needed that can certify the *Toxoplasma* status of a meat product, animal or farm
- International reference materials and laboratory certification programs are needed
- Further research is needed to develop new post harvest decontamination methods
Surveillance and monitoring of *Toxoplasma* in humans, food and animals (EFSA Journal (2007) 583, 1-64)

“Toxoplasmosis and agents thereof have to be reported by Member States according to their epidemiological situation (Directive 2003/99/EC). Despite this fact, no representative data are available for *Toxoplasma* in the EU, neither for humans nor for animals or food”
“As regards zoonoses and other agents where annual data is often scarce and reported by few Member States, data will only be reported every third year. This includes Toxoplasma, Q fever, Enterobacter sakazakii, histamine, staphylococcal enterotoxins and data on animal populations.”
Conclusions working group toxoplasmosis

Biohazard panel EFSA

- Characterize analytical methods in terms of sensitivity, specificity and other performance parameters. Absolute requirement for reference materials and reagents.
- Once the above is achieved: start monitoring on pre-harvest sector in sheep, goats, pigs and game.
**Toxoplasma safe meat**

Pre and post harvest procedures can lead to a toxoplasma-free meat label in analogy with approaches that have been used for salmonella free chicken meat.
Thank you for your attention