Diagnostic challenges in bovine and porcine cysticercosis

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Cysticercosis

- Infection with the metacestode larval stage (CYSTICERCUS) of taeniid tapeworms
- Adult tapeworms (Cestodes) are ribbon-like worms
  - Have a scolex, or head, a short neck, and a strobila, or segmented body formed of proglottids
  - Last proglottids are packed with eggs, detach from the body and leave the host via the stools or by active migration
- Final hosts can be humans, dogs, ...
- Two-host life cycle

Human taeniid tapeworms

- _Taenia solium_, _Taenia saginata_, _Taenia asiatica_

A. Ploos van Amstel et al. 2004, _J. Parasitol._ 90: 944-946
**Taenia spp - life cycle**

1. Onchospheres develop into cysticerci in muscle.
2. Humans infected by ingesting raw or undercooked infected meat.
3. Cattle (T. saginata) and pigs (T. solium) become infected by ingesting vegetation contaminated by eggs or gravid proglottids.
4. Eggs or gravid proglottids in feces and passed into environment.

**Taenia solium – life cycle**

- Neurocysticercosis
- Porcine cysticercosis
- Taeniasis

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*Taenia* spp – cysticerci in hart and muscles

https://biorender.com/icon/species/worms/taenia-solium-cysticercus

*Taenia* spp – cysticerci in the brain of a human and a pig (NCC, neurocysticercosis)

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*Taenia* spp – after ingestion by a final host

**Adult Taenia**

*Taenia solium*

*Taenia saginata*
**Taenia spp - Distribution**

*Taenia saginata* – beef tapeworm: cosmopolitan distribution

*Taenia solium* – pig raising/pork consuming developing countries
**Taenia spp - Distribution**

*Taenia asiatica* – confined to Asian countries

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**Taenia spp - importance**

- *Taenia saginata* and *Taenia asiatica*
  - **Disease burden low**: only intestinal taeniasis with no or little clinical consequences
  - **Economic burden high**: due to downgrading and condemning infected carcasses at meat inspection

- *Taenia solium*
  - **Disease burden high**: due to NCC
  - **Economic burden potentially high**: due to condemning infected carcasses at meat inspection, however, many infected pigs are not meat-inspected
Figure 11. Ranking of food-borne hazards, based on Disability-Adjusted Life Years at the global level, with 95% uncertainty intervals, 2010.

Notes: White dots indicate the median burden, black dots the inter-quartile range (IQR; 25th and 75th percentiles), red dots the 5th and 95th percentiles (5th and 95th percentiles). Note that the y-axis is on a logarithmic scale. Abbreviations: EPEC = Enteropathogenic Escherichia coli; ETEC = Enterotoxigenic E. coli; STEC = Shiga toxin-producing E. coli.

FIGURE 2. Global ranking of food-borne parasites using a multi-criteria ranking tool for scoring parasites, with weighting of scoring criteria based on criteria scores and weights elicited from expert meeting participants (Note: Trichinella spp. * includes Trichinella species except T. spiralis).
**Disease burden (DALY) of food-borne parasites according to WHO regions**

**Taenia solium - importance**

- Neglected zoonotic disease that causes a considerable disease burden on poor rural pig-keeping communities in developing countries
- Commonest cause of **acquired epilepsy** in endemic countries
- Concern in non-endemic areas due to international travelling
- *T. solium* is as a leading cause of deaths from food-borne diseases
  - 2.8 million DALYs
  - The total number of people with NCC between 2.56–8.30 million
**Taenia spp - control**

- Veterinary inspection of carcasses at the slaughterhouse - meat inspection regulations

- Sanitation – toilets and waste water treatment

- Hygiene

- Awareness – Health education

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**Taenia saginata - control**

- **Bovine → Human**
  
  Control of taeniasis by preventing infected meat to reach the consumer: meat inspection (EU directive 64/433/EEC) (all cattle > 6 weeks)

- **Human → Bovine**
  
  Regulations on use of domestic effluent and sewage sludge in agriculture (no specific control measures of bovine cysticercosis)
**Taenia saginata - control**

Meat inspection

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**Taenia saginata – control - result**

- **Taenia saginata:** persistence of transmission in both industrialised and developing countries – causes?
  - Low sensitivity of carcass inspection
  - Spread of eggs through sewage – water treatment plants – rivers – flooding
  - Culinary habit of eating raw or undercooked beef
  - Unawareness of Taenia carrier on environmental contamination

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**Taenia solium** – control - result

- *Taenia solium*: high prevalence in poor rural areas in developing countries – reasons?
  - Free roaming of pigs
  - Open defecation
  - No meat inspection - low sensitivity of carcass inspection
  - Culinary habit of eating raw or undercooked pork (barbecue)
  - Unawareness on porcine cysticercosis
- Risk of human to human transmission outside endemic areas: urban and overseas areas

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**Diagnosis of cysticercosis**

- Veterinary public health: meat safety
- Epidemiological studies
- Monitoring of control/intervention programs

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Bovine cysticercosis

Diagnosis
- Carcass inspection
- Carcass inspection +
- Dissection of predilection sites
- Full carcass dissection
- PCR and immunohistochemistry
- Serological methods
Carcass inspection


- Bovines > 6 weeks
- Part of standard carcass inspection procedures
Carcass inspection

- Inspection of the predilection sites
  - Visual inspection of the carcass surface and of the tongue, diaphragm and oesophagus
  - Examination of the external masseters, in which 2 incisions must be made, and the internal masseters, 1 incision
  - Visual inspection and incision of the heart

Findings: cysticerci

(a) viable  (b) degenerated  (c) calcified
Carcass inspection

- Regulation (EC) 854/2004 Chapter IX Specific hazards

- In addition, specific serological tests may be used
  - Incision of the masseters at post-mortem inspection is not compulsory when a specific serological test is used

- Meat infected with cysticercus is to be declared unfit for human consumption

- However, when the animal is not generally infected, the parts not infected may be declared fit for human consumption after having undergone a cold treatment

Carcass inspection

- Performance of carcass inspection
  - Known low sensitivity (especially in light infections)
    - Estimated between 10 and 30 %
    - EIDRUC (Belgium) project: sensitivity only 0.54 %!
  - Problems of objectivity/specificity?

- Low sensitivity, part of the problem of persistence of bovine cysticercosis
Carcass inspection +

- Carcass inspection + additional cuts in the heart muscle
- No further mutilation of the carcass
- No microbiological risk for meat

- Switzerland: detection of cysticerci X 2

- Belgium: “does not increase the sensitivity of the technique sufficiently to be considered profitable”

Dissection of the predilection sites

- Slicing predilection sites < 0.5 cm cuts
- Not feasible in the daily practice

- In Belgium: 25% of carcasses negative at meat inspection had cysticerci when totally dissecting predilection sites

- On average, around 23% of cysticerci are in predilection sites
Full carcass dissection

- Slicing entire carcass < 0.5 cm cuts
- Not feasible in the daily practice, experimental studies
  - Experimental infections
  - Vaccine experiments
- Gold standard technique
- High cost and labour intensive

Confirmatory tests

- Histology
- Immunohistochemical studies (B158 MoAb marker)
- Molecular methods
Serology

- Mentioned in Regulation (EC) 854/2004
- No commercial test available
- Many in-house tests
- Based on detection of specific antibodies or circulating antigens
- Mostly in ELISA format

Serology: Antibody versus antigen detection

- **Antibodies**
  - *indirect test*
  - Measures current infection and past infection, exposure

- **Antigens**
  - *Direct test: capturing circulating excretory/secretory (ES) antigens*
  - Measures viable infections only
Serology: Antibody Detection

- Antibody detection requires the use of specific antigens
  - Crude antigens
    - *Taenia saginata* crude cyst antigen, cyst fluid, ES
    - Heterologous antigens: *Taenia crassiceps*
  - Purified antigens
  - Recombinant antigens
  - Synthetic peptides

Serology: Antibody Detection

- Difficulties related to Ab detection
  - Antigen production
  - Validation! Full carcass detection as a gold standard
  - For most tests, Se and Sp calculated on not fully characterised samples: bias
  - Measure of exposure or past infection: validity?
  - Format: ELISA → quick test or high throughput test?
**Serology: Antibody Detection: examples**

- **Ab-ELISA based on ES Ag**
  - In a study in Switzerland
    - se 82% and sp 96% (Eichenberger et al. 2013)
  - The same test used in Belgium
    - se 14% and sp 93% (Jansen et al., 2018)

**Serology: Antigen detection**

- Antigen detection based on capturing circulating antigens by monoclonal antibody-based antigen ELISA
  - HP10 Ag-ELISA (Harrison et al., 1989)
  - B158/B60 Ag-ELISA (Brandt et al; 1992; Van Kerckhoven et al., 1998; Dorny et al., 2000)
- Detection of viable cysticerci only
Serology: Antigen detection

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<td>No. of cysticerci</td>
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<td>57</td>
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<td>0</td>
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<td>Establishment rate</td>
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<td>No. of viable cysticerci</td>
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<td>31</td>
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<td>Ag-ELISA RATIO</td>
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<td>24.3</td>
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Experimental infections: Ag-ELISA

![Antigen ratio post infection graph](image)
**Serology: Antigen detection**

- EIDRUC Belgium: test performances estimates
- B158/B60 Ag-ELISA:
  - Se: 48% (for detection of viable cysticerci)
  - Sp: 98%

Prevalence MI: 0.23%

Low Se of MI: 0.54%

Prevalence based on EIDRUC study: 30 - 40%
Long term effect of introducing Ag-ELISA as an alternative of meat inspection (Model, prediction, Belgian situation over a 10 years period)

Alternative serological diagnosis of bovine cysticercosis

- Cost of test
- Practical implementation
- Who will pay?
- Invest in an imperfect test?
- Initially high numbers of carcasses positive! High cost, loss for farmers
- Invest in a disease of low public health importance?
Porcine cysticercosis

Diagnosis of porcine cysticercosis

- Identifying infected pigs: elimination of infected animals from the food chain
- Indicator of exposure (proxy for human exposure)
- Measuring effect of intervention (sentinels)
Porcine cysticercosis

- Tongue inspection
- Carcass inspection
- Full carcass dissection
- Partial carcass dissection
- PCR and immunohistochemistry
- Serological methods

Diagnosis of cysticercosis in pigs: Tongue inspection

- Tongue inspection or palpation
- Simple, specific test

Taenia solium cysticerci on a pig’s tongue

Useful tool for rapid assessment of hot spots
Diagnosis of cysticercosis in pigs: Tongue inspection

- Performances:
  - Se 21%, sp 100% (Zambia, Dorny et al., 2004)
  - Se 8%, sp 80% (South Africa, Krecek et al., 2008)
- Pigs with cysts on tongue often go to illegal market, alternative circuit

Diagnosis of cysticercosis in pigs: Carcass inspection

- Inspection (and incision) of predilection sites at slaughter
- Simple
Diagnosis of cysticercosis in pigs: Carcass inspection

- Performances:
  - $\text{se} 22\%$, $\text{sp} 100\%$ (Zambia, Dorny et al., 2004)
- “Porcine cysticercosis”: can be caused by *T. solium*, *T. asiatica* and *T. hydatigena*: confusion in reporting
- Many pigs slaughtered in rural areas are not inspected

Diagnosis of cysticercosis in pigs: Full carcass dissection

- Slicing entire carcass < 0.5 cm cuts
- Not feasible in the daily practice, experimental studies
  - Experimental infections
  - Control studies
- Gold standard technique
- High cost and labour intensive
Diagnosis of cysticercosis in pigs: Full carcass dissection

- Challenges:
  - Cysticerci can be anywhere: also in organs (liver, lungs, spleen, brain, ...)
  - Size of cysticerci varies: some are very small
  - Cysticerci in liver to be differentiated from cysticerci of *T. asiatica*, *T. hydatigena* and from milk spots
Diagnosis of cysticercosis in pigs: **Partial carcass dissection** (Lightowlers et al., 2016)

- Dissection of only the tongue, masticatory muscles and heart
- 31 of the 38 (81%) naturally infected animals were identified as having cysts in these muscles
- relatively sensitive and highly specific method for diagnosis of porcine cysticercosis

Diagnosis of cysticercosis in pigs: **immunodiagnosis**

- **Benefits:**
  - Ante-mortem diagnosis
  - More sensitive and practical than tongue palpation
  - Used in prevalence and community-based surveys and intervention studies
Diagnosis of cysticercosis in pigs: immunodiagnosis

- Tests developed in humans adapted for pig samples
  - **Ab detection**: EITB, Ab-ELISA
  - **Ag detection**: Ag-ELISA (HP10 and B158/B60 Ag-ELISA)

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Diagnosis of cysticercosis in pigs: immunodiagnosis

**Ab-detection**
- Several antigens tested (crude, purified, recombinant) in different formats (ELISA, EITB)
- Some commercially available kits for humans can be adapted for use on pig sera
Diagnosis of cysticercosis in pigs: **immunodiagnosis**

**Ab detection**

- Same issues as for bovines: detection of current and past infections, exposure, aborted infections
- Transient antibodies
- Maternal antibodies may persist for several months in piglets born to *T. solium* infected sows
- Sensitivity low in pigs with low levels of cyst burdens
- Specificity? cross reactions with cysticerci of *T. hydatigena* and *T. asiatica*

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**Diagnosis of cysticercosis in pigs: immunodiagnosis**

**Ab detection**

- Test performance in papers often biased because of selection of samples and lack of gold standard
- Bayesian model: → true prevalence
  - Zambia: Ab-ELISA, crude metacestode *T. crassiceps* antigen: se 36%, sp 92%
  - South Africa: EITB: se 49%, sp 84%
Diagnosis of cysticercosis in pigs: **immunodiagnosis**

**Ab detection**

- **EITB**: Western Blot format, 7 lentil lectin purified glycoproteins (native antigen)
- **EITB**: first reports on diagnostic performance in pigs; se and sp close to 100%

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**Recent studies** (Jayashi et al., 2014; Gomez-Puerta et al., 2019)

- The GP50 band cross-reacts with *T. hydatigena* in pigs
- Using EITB reactivity to ≥1 band as a cut-off point for the assay, se 89% and sp 48%
- Reactivity of ≥3 bands provided the best trade-off, se 78% and sp 76%
Diagnosis of cysticercosis in pigs: **immunodiagnosis**

**Ag detection**

- Antigen detection based on capturing circulating antigens by monoclonal antibody-based antigen ELISA’s
- Same methods as in cattle: genus specificity of tests
- HP10 Ag-ELISA (Harrison et al., 1989)
- B158/B60 Ag-ELISA (Brandt et al.; 1992; Van Kerckhoven et al., 1998; Dorny et al., 2000). Commercialised by apDia

- Detection of viable cysticerci only

**Test performances:**

- **Zambia** (Dorny et al., 2004)
  - B158/B60 Ag-ELISA: se 87%, sp 95%

- **South Africa** (Krecek et al., 2008, 2011)
  - B158/B60 Ag-ELISA: se 63%, sp 87%
  - HP10 Ag-ELISA: se 70%, sp 66%
Diagnosis of cysticercosis in pigs: **immunodiagnosis**

**Ag detection**

- **Important issues**
  - **Cross reactivity**
    - Due to genus specificity of tests
    - Big problem in countries with high prevalence of *T. hydatigena* in pigs (SE Asia, Peru, Ecuador)
    - Situation in Africa less dramatic? Studies ongoing
    - Transient antigens?

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*Taenia hydatigena*
Immunodiagnosis of cysticercosis in pigs

Prospects

- Need for:
  - *T. solium* – specific tests
  - Pen-side tests

- Proteomic analysis of cysticercus antigens
- Improvement of the specificity of Ag-ELISA in pigs
  - Camelid-derived single-domain antibody fragments (nanobodies)

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Immunodiagnosis of cysticercosis in pigs

Prospects

- Use of new (CDC) tests for Ab-detection in pigs? (T24 recombinant antigen: WB → ELISA → Lateral Flow test)
- Development of pen-side tests
Conclusions

- *Taenia saginata* persisting, mainly because of low sensitivity of meat inspection:
  - *Need for a more sensitive test to detect bovine cysticercosis*
- *Taenia solium* most important food-borne parasite, endemicity in endemic countries
  - Currently pilot projects on control/elimination, using intervention tools such as, pig vaccination and treatment, health education and sanitation
  - *Need for pen-side test and T. solium specific test to detect porcine cysticercosis*

- Medical sector blaming veterinary sector not to do its job
- Cause of infection of animals are human tapeworm carriers: also other measures to be taken!

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