



**INSTITUTE
OF TROPICAL
MEDICINE**
ANTWERP



ISWAVLD2019

The 19th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (ISWAVLD) 2019
19th-22nd June 2019, Chiang Mai, Thailand

Diagnostic challenges in bovine and porcine cysticercosis

PIERRE DORNY

Department of Biomedical Sciences, Institute of Tropical Medicine

Faculty of Veterinary Medicine, Ghent University



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Cysticercosis

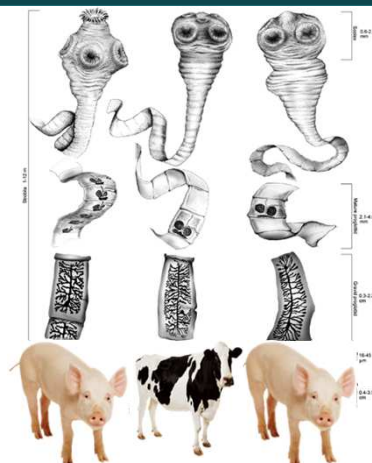
- Infection with the metacestode larval stage (CYSTICERCUS) of taeniid tapeworms
- Adult tapeworms (Cestodes) are ribbon-like worms
 - Have a scolex, or head, a short neck, and a strobila, or segmented body formed of proglottids
 - Last proglottids are packed with eggs, detach from the body and leave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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Human taeniid tapeworms

Taenia solium *Taenia saginata* *Taenia asiatica*

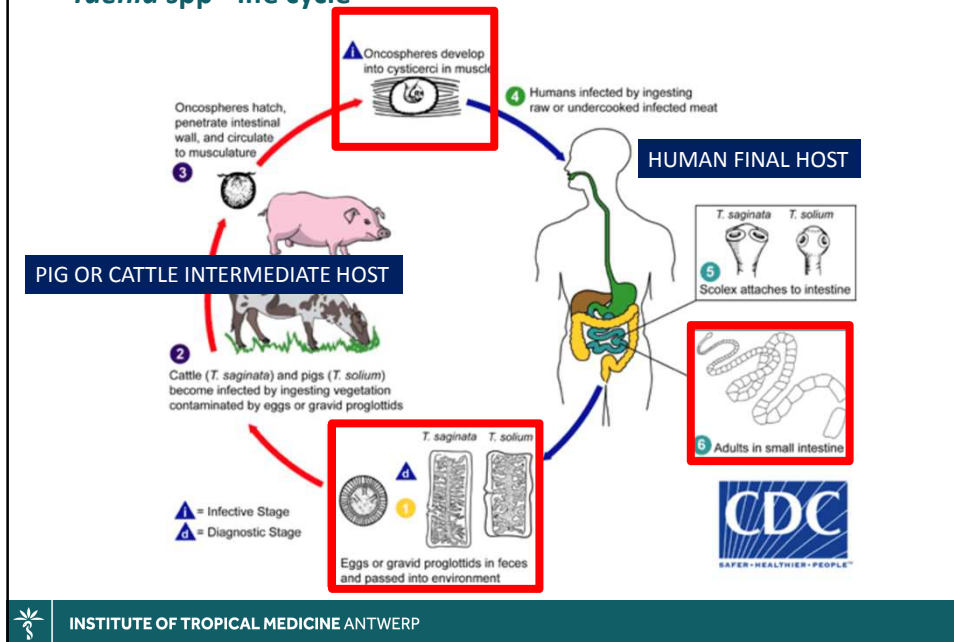


A. Flisser et al. 2004, J. Parasitol. 90, 914-916

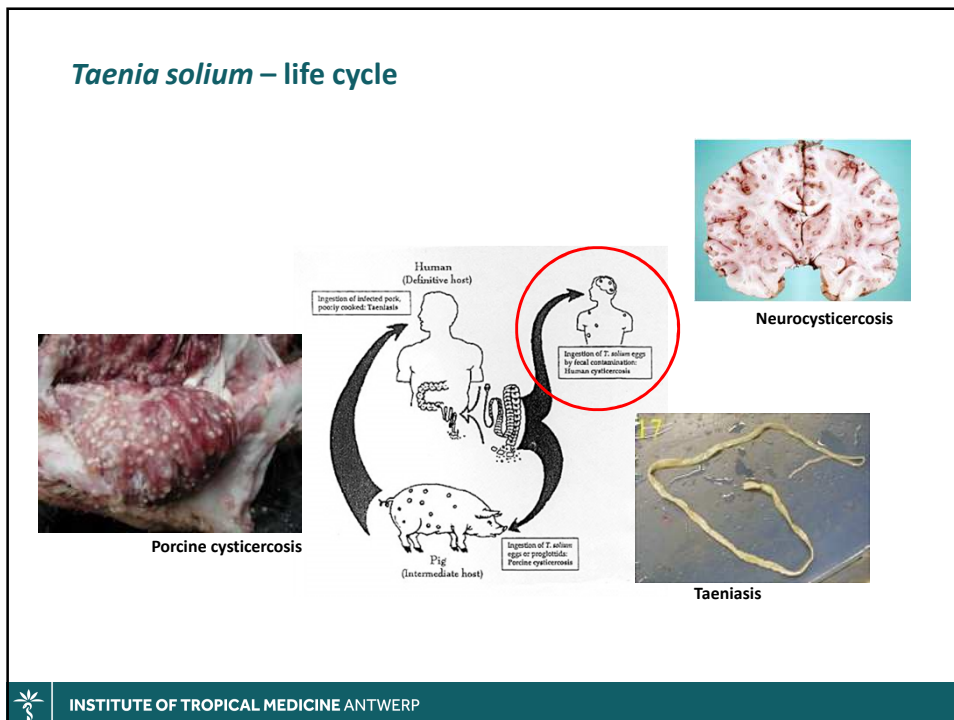


INSTITUTE OF TROPICAL MEDICINE ANTWERP

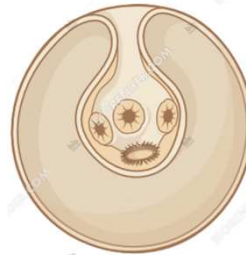
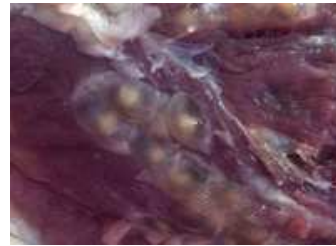
Taenia spp - life cycle



Taenia solium – life cycle



Taenia spp – cysticerci in hart and muscles

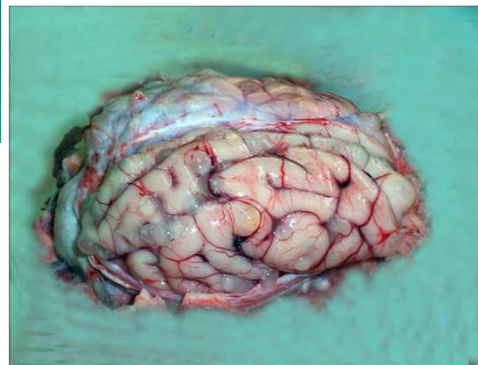


<https://biorender.com/icon/species/worms/taenia-solium-cysticercus>



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Taenia spp – after ingestion by a final host



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Adult Taenia



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Taenia spp - Distribution

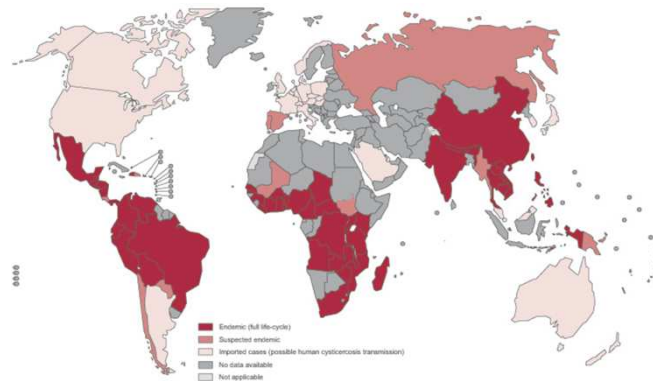
Taenia saginata – beef tapeworm: cosmopolitan distribution



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Taenia spp - Distribution

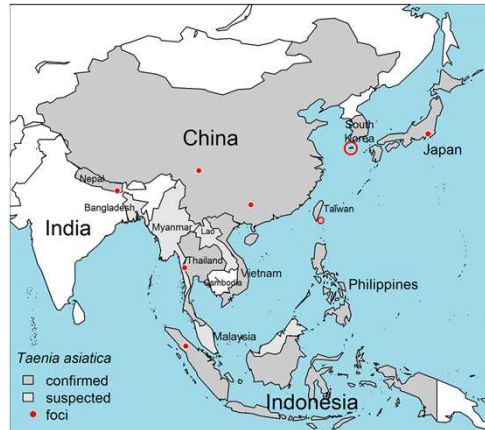
Taenia solium – pig raising/pork consuming developing countries



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Taenia spp - Distribution

Taenia asiatica – confined to Asian countries



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

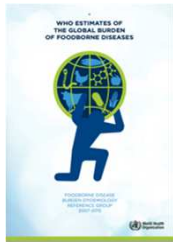
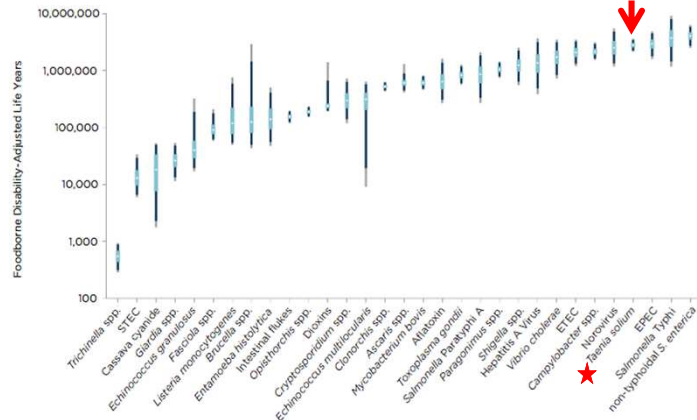


Figure 11. Ranking of foodborne hazards, based on Disability-Adjusted Life Years at the global level, with 95% uncertainty intervals, 2010.



Notes: White dots indicate the median burden, black boxes the inter-quartile range (50% UI), black lines the 5 and 95 percentiles (90% UI) and gray lines the 2.5 and 97.5 percentiles (95% UI). 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.

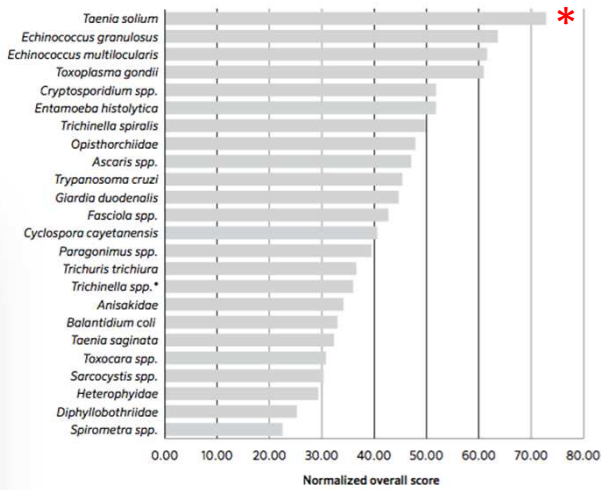
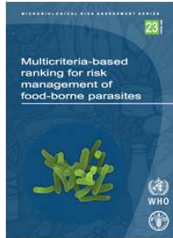
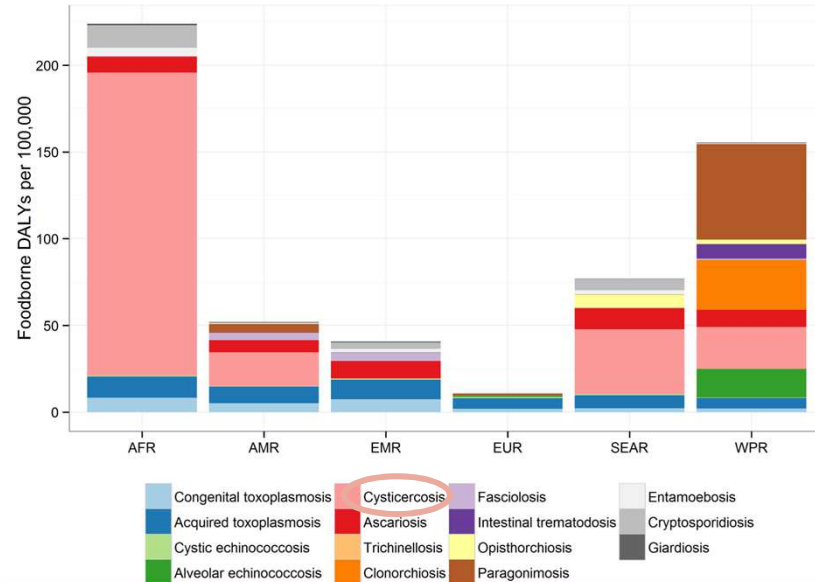


FIGURE 2. Global ranking of food-borne parasites using a multicriteria ranking tool for scoring parasites, with weighting of scoring criteria based on criteria scores and weights elicited from expert meeting participants (Note: Trichinella spp.* includes Trichinella species except T. spiralis).

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**



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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)



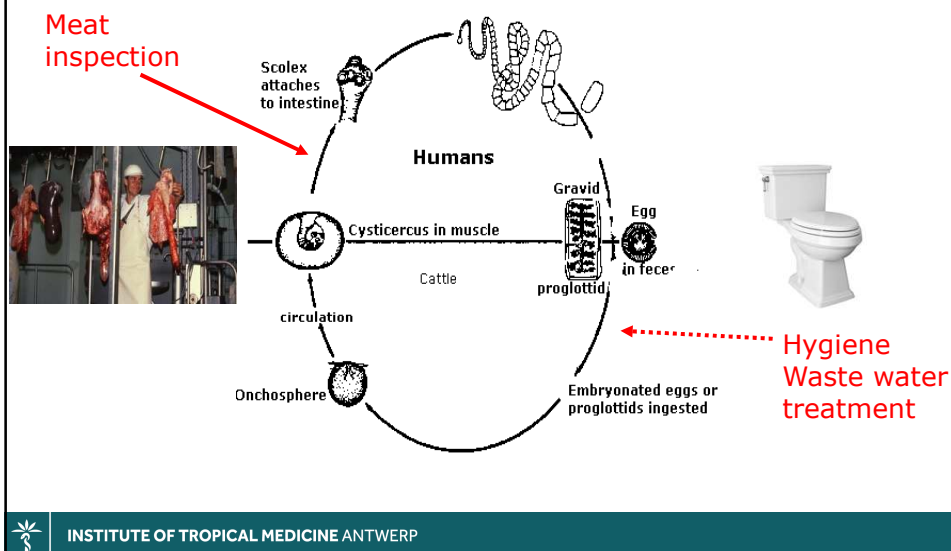
■ Human → Bovine

Regulations on use of domestic effluent and sewage sludge in agriculture (no specific control measures of bovine cysticercosis)



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Taenia saginata - control



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

Taenia solium – control - result

- *Taenia solium*: high prevalence in poor rural areas in developing countries – **causes?**
 - 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

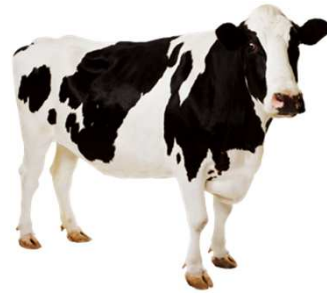


Diagnosis of cysticercosis

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



Bovine cysticercosis

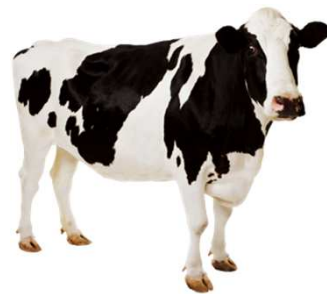


INSTITUTE OF TROPICAL MEDICINE ANTWERP

Bovine cysticercosis

Diagnosis

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Carcass inspection



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Carcass inspection

[Regulation \(EC\) 854/2004](#) laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

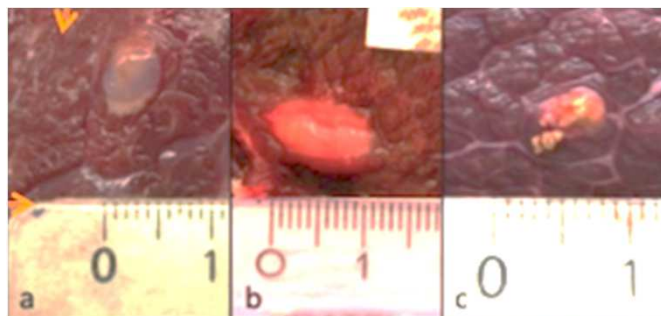
Carcass inspection

Findings: cysticerci

(a) viable

(b) degenerated

(c) calcified



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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”



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Confirmatory tests

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



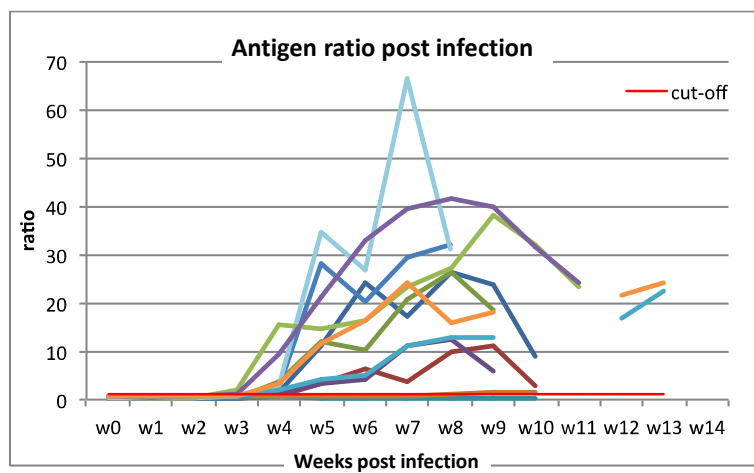
Serology: Antigen detection

	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12
No. of eggs administered	30,000	30,000	15,000	15,000	7500	7500	7500	7500	3500	3500	30,000	30,000
No. of cysticerci	312	138	748	57	1	0	99	81	38	36	92	94
Establishment rate	1.04	0.46	4.99	0.19	0.01	0	1.32	1.08	1.09	1.03	0.31	0.31
No. of viable cysticerci	177	93	580	31	1	0	72	67	38	34	90	89
Ag-ELISA RATIO	24	11	18.6	6	1.2	0.6	23.4	24.3				



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Experimental infections: Ag-ELISA



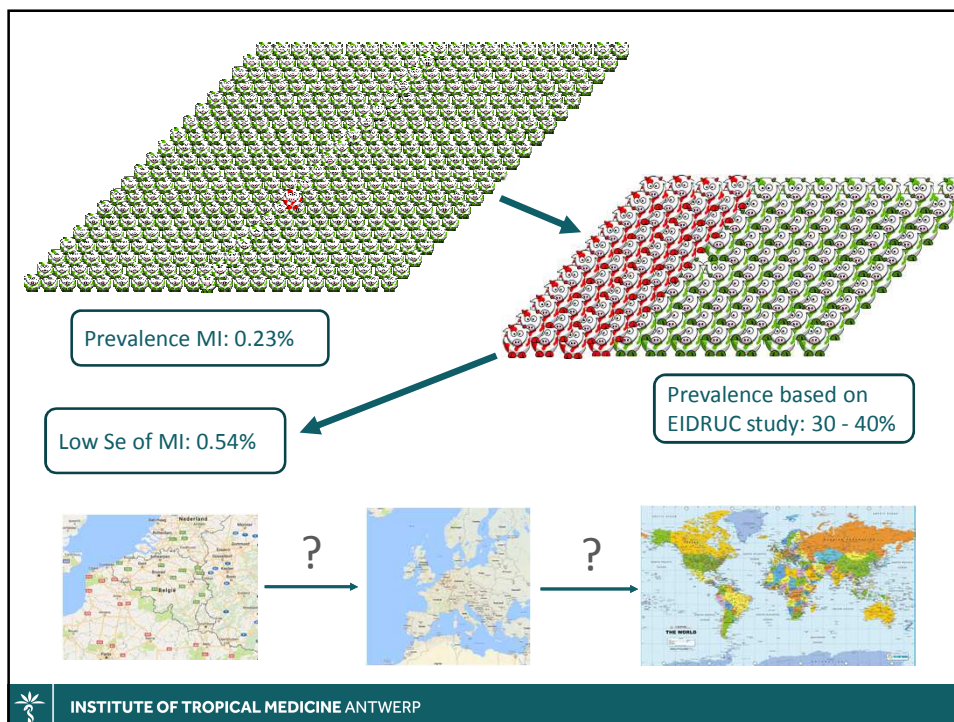
INSTITUTE OF TROPICAL MEDICINE ANTWERP

Serology: Antigen detection

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

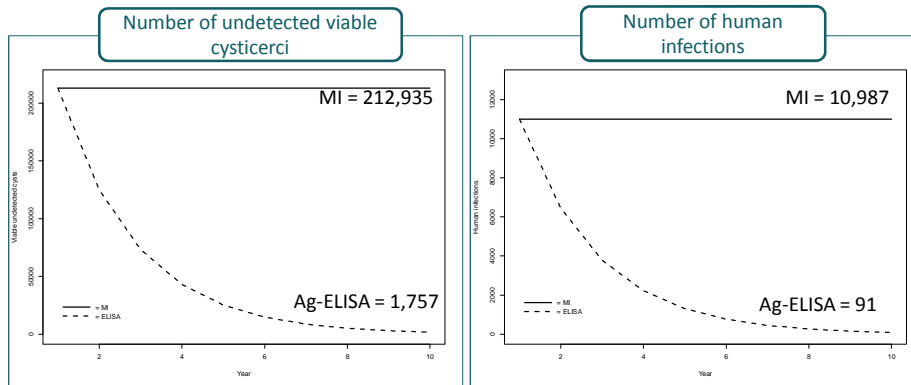


INSTITUTE OF TROPICAL MEDICINE ANTWERP



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Long term effect of introducing Ag-ELISA as an alternative of meat inspection (Model, prediction, Belgian situation over a 10 years period)



INSTITUTE OF TROPICAL MEDICINE ANTWERP

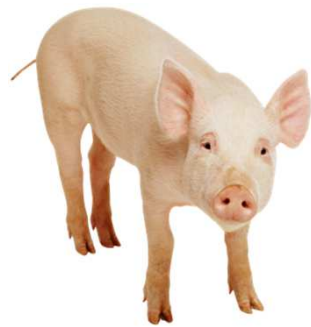
Alternative serological diagnosis of bovine cysticercosis

- 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?



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Porcine cysticercosis



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Diagnosis of porcine cysticercosis

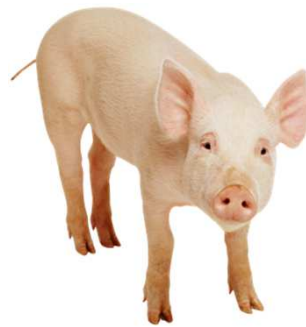
- Identifying infected pigs: elimination of infected animals from the food chain
- Indicator of exposure (proxy for human exposure)
- Measuring effect of intervention (sentinels)



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Porcine cysticercosis

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Diagnosis of cysticercosis in pigs: Tongue inspection

- Tongue inspection or palpation
- Simple, specific test



Useful tool for rapid
assessment of hot spots



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Diagnosis of cysticercosis in pigs: **Carcass inspection**

■ Inspection (and incision) of predilection sites at slaughter

■ Simple



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Diagnosis of cysticercosis in pigs: **Carcass inspection**

- Performances:
 - se 22%, 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



Full carcass dissection



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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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)



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*



Diagnosis of cysticercosis in pigs: immunodiagnosis

Ab detection

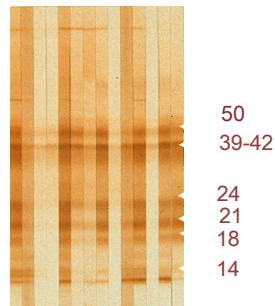
- Test performance in papers often biased because of selection of samples and lack of gold standard
- Bayesian model: → true prevalence
 - Zambia: Ab-ELISA, crude metacestode *T. crassiceps* antigen: se 36%, sp 92%
 - South Africa: EITB: se 49%, sp 84%



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%



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Diagnosis of cysticercosis in pigs: immunodiagnosis

Ab detection

- 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%



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Diagnosis of cysticercosis in pigs: immunodiagnosis

Ag detection

- 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%



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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?



INSTITUTE OF TROPICAL MEDICINE ANTWERP

Taenia hydatigena



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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)

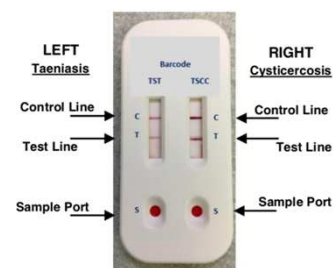


INSTITUTE OF TROPICAL MEDICINE ANTWERP

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



INSTITUTE OF TROPICAL MEDICINE ANTWERP

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!*



INSTITUTE OF TROPICAL MEDICINE ANTWERP



INSTITUTE
OF TROPICAL
MEDICINE
ANTWERP

Pierre Dorny

pdorny@itg.be