First 2016 Cystinet Working Group and Management Committee Meeting

2-3 June 2016

Lecture theatre Duclaux
Institut Pasteur
28 rue Dr Roux, 75015 Paris
Welcome to Paris

Local organizing committee

Département des Parasites et des insectes vecteurs

&

Direction International

Institut Pasteur, Paris

Paralim Team

JRU BIPAR

NRL Foodborne Parasites

OIE Collaborating Centre for Foodborne Zoonotic Parasites
PROGRAMME
**Working Group Meeting:** Amphitheater Duclaux

09:00 - 10:00 Plenary session

- 09:00 Reception of participants
- 09:15 Welcome
  
  M. Jouan Directeur International Institut Pasteur
  
  P. Boireau Directeur Laboratoire Santé Animale, Anses
  
  B. Polack (ENVA) & R Jambou (Institut Pasteur) local organizers
- 09:30 Presentation of the COST action TD1302 CYSTINET and general progress, introduction Year 3 WBP (S. Gabriël) - STSM presentation (B. Soba)
- COST rules: a quick reminder (A. Vlaminck)

10:00-10:15 coffee break

10:15 - 11:15 Short separate Working Group session

(briefing of activities since last meeting)

11:15-12:15 Plenary session of all working groups

- WG1. Brecht Devleesschauwer & Alberto Allepuz

12:15 - 13:00 Lunch (Carrel 3 underground level, social building)
13:00 - 17:00 Plenary session of all working groups cont. (with coffee break)
  o WG1 cont if needed
  o WG2. Andrea Winkler & Teresa Garate & Pierre Dorny
    - *Ring Trial to detect anti-Cysticercus cellulose IgG in human sera.* M.A. Gomez-Morales

15:00-15:15 coffee break

  o WG3. Maria Vang Johansen & Elias Papadopoulos
    Abstract presentations:
    - *Full carcass dissections of naturally Taenia solium infected pigs: new insights.* E. Mwape
    - *Identification of practices of home slaughtering of livestock and official meat inspection management in slaughterhouses in the different EU countries to assess the risk of T. solium and T. saginata.* A. van Roon

**Management Committee Meeting**
17:00 - 18:00 Only for MC members

**CYSTINET Dinner (restaurant LE PROCOPE)**
20:00  13 rue de l'Ancienne Comédie - PARIS 6ème, Métro Odéon
Working Group Meeting: Amphitheater Duclaux

09:00 – 10:30 Plenary session: Amphitheater Duclaux

Presentations of invited speakers

- P. Torgerson, Vetsuisse Faculty, Zurich, Switzerland. *The global burden of Neurocysticercosis*
- A. Lucianez, Pan American Health Organisation. *Control of Taenia solium in Latin America*
- S. Ramiandrasoa, Cysticercosis 's National country coordinator, Ministry of Public Health, Madagascar. *Current situation of the fight against Taeniosis/cysticercosis in Madagascar*
- Men-Bao Qian, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, People’s Republic of China. *Control of Taenia solium in China*

10:30-10:45: coffee break

Two parallel sessions:

1. **Session abstract presentations**
2. **Closed session on control of Taenia solium in endemic areas** (on invitation only)

11:00-13:00 **Abstract presentations**: Amphitheater Duclaux

- *Evaluation of plasma n-glycome as potential biomarker for neurocysticercosis*. T. Garate
- *Sensitivity and specificity of lamp assays for taenia species detection*. T. Garate
- *Comparison of diagnostic tools for diagnosis of Taenia solium cysticercosis in naturally infected pigs*. C. Laforet
- **Socio-economic impacts of Taenia solium cysticercosis and neurocysticercosis in eastern Zambia.** E. Hobbs
- **Evaluation of three recombinant proteins for the serodiagnosis of neurocysticercosis (NCC).** A. Hernandez-Gonzalez

13:00-14:30 lunch: (Carrel 3 underground level, social building)

14:30-15:30 Museum visit

15:30-16:30 **Abstract presentations cont.:** Amphitheater Duclaux

- **Costs involved with implementation of different post-mortem detection techniques for bovine cysticercosis in Belgium.** F. Jansen
- **Teniosis and some other foodborne parasites in Hungary regarding 10 years period.** A.J. Laki
- **Seroprevalence of porcine cysticercosis in north-western Romania.** V. Cozma
- **Porcine Cysticercosis in Serbia.** B. Bobic
- **Diagnosis of viable Taenia metacestodes through detection of the secreted HP10 antigen.** M. Parkhouse
- **Mapping areas contaminated by Taenia solium eggs: a spatial multi-criteria evaluation approach in Madagascar.** V. Porphyre

11:00-13:00 **Parallel Closed Session (on invitation):**
Salle Bordet Room – Building Metchnikoff

**Taenia solium control in endemic areas:** The aim of this specific session is to move the dialogue forward from the 2014 WHO informal consultation and 2015 WHO diagnostics meeting as we strive to achieve the goals set by the road-map for control of neglected tropical diseases. *Moderated by E. Fèvre.*

13:00-14:00 lunch: (together with other group)

14:00-16:30 **Parallel Closed session: Taenia solium control in endemic areas cont.**
(Bordet Room Building Metchnikoff)

16:30: **closing of the meeting and Coffee**
Ring Trial to detect anti-

*Cysticercus cellulose* IgG in human sera

Maria Angeles Gómez-Morales\textsuperscript{1,2}, Laura Camoni\textsuperscript{2}, Joachim Blocher\textsuperscript{3}, Marco Amati\textsuperscript{1,2}, Patrizio Pezzotti\textsuperscript{2}, Edoardo Pozio\textsuperscript{1,2}, Ring Trial participants\textsuperscript{4}

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\textsuperscript{2} Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
\textsuperscript{3} University Medical Center Göttingen, Göttingen, Germany
\textsuperscript{4} Ring Trial Participants: Claire Alexander, SPDRL Glasgow Royal Infirmary, Glasgow, United Kingdom; Herbert Auer, Dept. Med. Parasitology, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria; Silvana Bello, Instituto de Higiene e Medicina Tropical, Universidade NOVA de Lisboa, Lisbon, Portugal; Zorica Dakic, Parasitological Laboratory at Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade, Serbia; Pierre Dorny, Institute of Tropical Medicine, Department of Biomedical Sciences, Veterinary Helminthology Unit, Antwerpen, Belgium; Teresa Gárate, Instituto de Salud Carlos III, Centro Nacional de Microbiología Parasitología, Majadahonda, Madrid, Spain; Ronan Jambou, Institut Pasteur de Côte d’Ivoire, Abidjan, Côte d’Ivoire; Titia Kortbeek, RIVM Bilthoven Centre IDS, Bilthoven, The Netherlands; Ingrid Reiter-Owona, Institute of Medical Microbiology, Immunology and Parasitology, University Clinic Bonn, Bonn, Germany; Barbara Soba, Laboratory for parasitology, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

According to the program of the COST ACTION TD 1302 CYSTINET, European Network on Taeniosis/Cysticercosis, a Ring Trial to detect anti-*Cysticercus cellulose* IgG or *C. cellulosae* circulating antigens in human sera has been organized by the European Union Reference Laboratory for Parasites and CYSTINET members. The aim of the collaborative study was to determine the performance of the tests used in the European Union and associated countries. Participating laboratories could use any serological test based on the detection of anti-*C. cellulose* IgG or *C. cellulosae* circulating antigens, preferably the test/s routinely used in their laboratories. Eleven laboratories from Austria, Belgium, France, Germany, Italy, the Netherlands, Portugal, Serbia, Slovenia, Spain, and United Kingdom, agreed to participate. The panel of sera consisted of 12 individual human serum samples: 2 from blood donors, one from a person with cystic echinococcosis in the liver, one from a person with cervical adenocarcinoma, and eight from persons with a final diagnosis of neurocysticercosis. The samples were dispatched frozen to the participating laboratories by an international courier in March 2016. Eleven different tests, which detect anti-*C. cellulose* IgG, were used by eight laboratories. One laboratory did not send the results. All tests but one reached 100% specificity. The highest sensitivity (91.7%) was shown by only one test, which reached the best accuracy (0.9583, 95% IC 0.85-0.99). Out of the two tests used to detect *C. cellulosae* circulating antigens by three laboratories, one showed a 100% specificity, 62.5% sensitivity, and 0.81 (95% IC 0.51-0.99) accuracy.
Full carcass dissections of naturally *Taenia solium* infected pigs in Zambia: unexpected findings


CYSTISTOP is a study in which the implementation of combined, highly intensive interventions (health education, human mass drug administration, pig treatment and pig vaccination) aimed at elimination of *Taenia solium* as well as the implementation of a less intensive intervention (health education, pig treatment) aimed to control *T. solium*, will be evaluated in rural communities of Sinda and Katete district in the Eastern Province of Zambia.

In the framework of this study, a total of 66 randomly selected pigs of slaughter age were dissected to obtain baseline prevalence information. Dissections were done on full carcasses, including all the organs. Total cyst counts were recorded and collected cysticerci identified morphologically and confirmed molecularly using PCR-restriction fragment length polymorphism (PCR-RFLP).

Key findings were the high occurrence of *T. solium* infected pigs (37 animals, 56%) and the presence of *T. solium* cysts in the livers of 10 of the 37 infected animals (27%). A high proportion of infected/positive carcasses (29/37; 78.3%) had low infection levels (15/37, 40.5% with <10 cysts and 14/37, 37.8% with 11-50 cysts). Another interesting finding was the presence of very small (viable) cysts throughout the carcass, which could be easily missed at dissection, possibly leading to underreporting. In six carcasses (9%), both *Taenia hydatigena* and *T. solium* were identified. No carcasses were identified with only *T. hydatigena*.

Visceral organs are usually not considered when carcass dissections are used as a diagnostic tool to detect (levels of) *T. solium* infections in pigs, as the cysticerci normally establish in the muscles, subcutaneously and in the central nervous system. Our results, indicating the presence of cysticerci in 27% of the livers of infected animals, demonstrate the need to review the methods of full carcass dissections.

These are important findings for transmission, monitoring and diagnostics.
Home slaughter practices of livestock and official meat inspection management in slaughterhouses in the different EU countries to assess the risk of *T. solium* and *T. saginata*.

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Background. CYSTINET WG3 focuses on control and prevention of *Taenia solium* and *T. saginata*. Although home slaughtering of pigs is allowed in the EU, during the Cystinet meeting in Rome (2015) it was indicated by CYSTINET WG 3 members that home slaughtering is a potential risk factor for the transmission of the parasites. The latter could potentially be true, especially if meat inspection is not conducted during home slaughtering. The aim of this study was to identify home slaughtering practices of livestock and official meat inspection management in slaughterhouses in the different EU countries.

Materials & methods. An online questionnaire was developed to ask for home slaughtering practices in EU countries, aiming to assess possible risks of uncontrolled home slaughtering practices regarding *T. solium* and *T. saginata* and to compare meat inspection management in the EU regarding the control of *T. solium* and *T. saginata* in slaughterhouses. The online questionnaire was forwarded by CYSTINET members from different countries to people knowledgeable on the topic in their country. The questionnaire included questions about whether home slaughtering of pigs and cattle was allowed in their country and practiced, questions on the European- and national regulation on home slaughtering of pigs and cattle, and official meat inspection of swine and bovine at slaughterhouses.

The data collected was analyzed using Microsoft Excel and SPSS.

Results. To date, the questionnaire was filled out by 18 experts from 12 different countries. Respondents were veterinarians, teachers, researchers and inspectors from universities, veterinary laboratories and competent authorities on meat inspection. Preliminary results showed that home slaughtering of pigs was allowed in 11 out of 12 questioned countries, mainly for private domestic consumption but in some countries also for placing small quantities of meat on the market. Home slaughtering of cattle was allowed in 6 out of 12 questioned countries, mainly for private domestic consumption. However, some respondents indicated that home slaughtering of cattle was practiced in their country despite being illegal. Whether official meat inspection was mandatory during home slaughtering varied per country. Future research should focus on how home slaughtering and meat inspections are practiced.
Evaluation of plasma n-glycome as potential biomarker for neurocysticercosis

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BACKGROUND. Human neurocysticercosis (NCC) is a heterogeneous pathology. Clinical manifestations (from asymptomatic to a severe intracranial hypertension) vary according to number, size, stage and location of cysts, intensity of host immune response and parasite genotype. Although diagnosis tools and treatments have improved during the last decades, the prognostic of the patients is highly variable; some of them will completely respond to the first therapeutical cycle while in others, parasites will persist in spite of numerous treatment courses and severe complications can be developed. Therefore, the availability of new markers, e.g. related to disease prognosis, different NCC pathological forms, response to cysticidal drugs and to anti-inflammatory treatments, etc, would help clinician in the patient management and its follow-up.

In recent years, plasma N-glycans have emerged as biomarkers for health and disease. During the development of disease, changes in cellular glycosylation are observed, indicating that alterations of the glycome occur in extracellular fluids as well as in plasma/serum and could therefore serve as disease progression biomarkers.

The aim of this work was to identify new potential glycan biomarkers in NCC.

MATERIALS AND METHODS. Plasma was collected from 80 NCC patients diagnosed with active or inactive cerebral cisticercosis (40 HP10 positive and 40 HP10 negative), 12 patients with other noninfectious neuropathologies and 60 age-matched healthy controls (de españa?). Total plasma N-glycome was measured by comprehensive and quantitative analysis by UPLC after deglycosylation and labeling with 2-AB.

RESULTS. From each plasma sample, 38 individual glycans and 17 different traits (antennae, syalylation, galactosylation, fucosylation, high-mannosylation) were evaluated.

CONCLUSIONS. We demonstrated, for first time whole plasma glycan profiling of NCC patients in comparison to non-infectious and showed that some individual glycans exhibit differential variation in their level in both groups when compared among each other and with the control population.
Sensitivity and specificity of LAMP assay for *Taenia* species detection.

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BACKGROUND: In a previous work we established the operation of a loop-mediated isothermal amplification (LAMP) assay previously described to amplify DNA from *Taenia saginata*, *T. solium* and *T. asiatica*. Additionally, a set of primers for LAMP assay to detect DNA from *T. hydatigena* was initially *in silico* selected. In this work, we have evaluated the sensitivity and specificity of the improved and standardized LAMP assays as well as the operation of the new designed LAMP assay for *T. hydatigena* detection.

MATERIALS AND METHODS: To determine the lower detection limit of the LAMP assays for *Taenia* species detection, genomic DNA from *T. saginata*, *T. solium* and *T. asiatica* serially diluted was subjected to amplification. The specificity of the LAMP assays for each species was tested against DNA from other *Taenia* species and also against other 16 DNA samples obtained from other parasites including several trematodes, nematodes and protozoa. For all trials our well-established LAMP reaction mixtures were used. We also evaluated the operation of the new set of primers for DNA amplification of *T. hydatigena* by testing a PCR using outer primers F3 and B3 and also by LAMP amplification.

RESULTS: The limit of genomic DNA detection for each *Taenia* species tested was as follows: *T. saginata*: 0.2 pg; *T. solium*: 2 pg and *T. asiatica*: 1 pg. When the panel of several DNA from other parasites were subjected to the specific LAMP assay for each *Taenia* species detection, amplicons were never obtained. PCR using outer primers F3 and B3 for detection of *T. hydatigena* amplified the 207 bp *in silico* expected fragment. We also obtained amplification results when testing DNA from *T. hydatigena* by using the designed LAMP assay. All LAMP amplification results could be visually observed by inspecting color change by naked eye and also on agarose gels electrophoresis.

DISCUSSION/CONCLUSION: The sensitivity and specificity of previously described and further improved LAMP assays for *Taenia* species have been established. A LAMP method for specific detection of *T. hydatigena* has been designed and is currently being standardized.

ACKNOWLEDGEMENTS: We thank Doctors Gabriel and Dorny for providing taeniids gDNAs, CYSTINET and RICET-RETIC.
Comparison of diagnostic tools for *Taenia solium* cysticercosis in naturally infected pigs

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Increasing efforts are done for the control and prevention of the neglected parasitic zoonosis, *Taenia solium* cysticercosis. The aim of this pilot study, conducted in the endemic region of Itasy, Madagascar, was to evaluate and compare five diagnostic tools for community diagnosis of *Taenia solium* cysticercosis in pigs in order to respond to the recommendations from the World Health Organisation (WHO) for the latest overall cysticercosis control strategy. A total of ten naturally infected and five control pigs were selected for the study based on tongue examination. Results from post-mortem examination were used as gold standard. Four diagnostic tools were evaluated, namely: ultrasound examination, serum Antigen and Antibody-ELISA and EITB. The following criteria: reliability (sensitivity and specificity), cost-efficiency, time and convenience were taken into account when comparing the tools. The commercial Ag-ELISA kit (ApDia Ltd., Belgium) provided the best results in terms of sensitivity (100%) and specificity (100%). Moreover it appeared to be quick, easy to use and requiring limited equipment. Ultrasound (FFsonic UF-4100 from Fukuda Denshi), conducted with a convex 3.5 Hz probe, proved to be a rapid non-invasive method, however, interpretation of the scanned images challenged the observer and low specificity (0%) results made it an unreliable diagnostic tool. In-house Ab-ELISA and EITB both performed well with high sensitivity (both 100%) and quite high specificity (80% and 75%, respectively) but the procedures were longer and more complex than with the commercial Ag-ELISA kit. In future, the commercial Ag-ELISA kit should be recommended as a fast, cost-efficient and reliable test for the diagnosis of *T. solium* cysticercosis in pigs.
Socioeconomic impacts of *Taenia solium* cysticercosis and neurocysticercosis in eastern Zambia


CYSTISTOP is an intervention study evaluating whether elimination or control of *Taenia solium* is the more cost-effective and locally acceptable option for sub-Saharan Africa. An additional aim of the study is to estimate the socioeconomic cost of *T. solium* cysticercosis infections in people and pigs, in a highly endemic region of Zambia.

Fifty percent of households in the study villages in the Katete and Sinda districts in eastern Zambia were selected randomly and exposed to two separate questionnaire surveys between 2015 and 2016. A human health questionnaire captured direct and indirect costs to the individual, arising from neurocysticercosis. A socioeconomic pig questionnaire captured data about pig herd demographics, costs related to pig-keeping, and both direct and indirect economic losses arising from porcine cysticercosis.

In total, 201 and 271 respondents completed the human and pig socioeconomic questionnaires, respectively. Of the human questionnaire respondents, 24/201 (11.9%) had reported seizure-like episodes, with 5/201 (2.5%) having sustained injuries during those episodes. 83/201 (41.3%) had experienced severe chronic headaches, 45/201 (22.4%) reported blurry vision, and 4/201 (2.0%) suffered blindness in one or both eyes. In total, 959 working days were lost due to these conditions over a five-year period.

From the pig socioeconomic questionnaire responses, 69.4% (188/271) of owners bought their pigs from within the village, and 95.2% (258/271) sold their pigs to traders. On average, pigs were bought at 7 months of age for approximately K90.00 ($9.00USD). Pigs were normally sold at approximately 24 months of age for an average of K240.00 ($24.00USD).

76.4% (207/271) of pig owners reported checking the pigs’ tongues for cysts at the point of sale, and 94.5% (256/271) reported being unable to sell tongue-positive pigs. Cysticercotic pigs were most commonly sold for 100Kw ($10.00USD), representing a loss of approximately 60% of the normal value of a sale-age pig.

The results indicate that *T. solium* causes significant social, economic and public health impacts on individuals living in endemic communities in Zambia. Economic losses arise from human infections, which prevent individuals from being able to work, and porcine infections, which reduce the sale value of pigs by up to 60%.
Evaluation of three recombinant proteins for the serodiagnosis of neurocysticercosis (ncc)

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BACKGROUND. Currently, the reference standard assay for the serodiagnosis of NCC is the lentil lectin-bound glycoproteins/enzyme-linked immunoelectrotransfer blot (LLGP-EITB) (Tsang et al. 1989). The main disadvantage of this technique is the complexity of obtaining and purifying the LLGP extract. This could be solved by replacing it with highly specific recombinant antigens from Taenia solium.

Based on previous studies (e.g. Hancock et al. 2006 and Ferrer et al. 2007), we selected and produced the recombinant T8B2 and T24H proteins and applied them to three diagnostic techniques: Western Blot (WB), ELISA and Luminex.

MATERIALS & METHODS. At CNM-ISCIII, these two sequences were expressed in a prokaryotic system and purified, resulting the following three recombinant proteins: T24H, GST-T24H and GST-T8B2. The proteins on the three techniques were tested against 149 sera from patients with NCC confirmed by image and serology, 40 sera from patients with other parasitic diseases and 130 sera from healthy individuals (clinical samples provided by CDC). The sensitivity and specificity for each antigen in WB were calculated by counting the number of true and false positive results. For the ELISAs and Luminex tests, the cutoff values, sensitivity and specificity were established by ROC curves.

RESULTS. All three antigens showed a high sensitivity in active NCC cases with multiple cysts and low sensitivity for cases of calcified lesions. WB was highest in specificity and in sensitivity. The recombinant T24H was the best diagnostic reagent in WB (100% sensitivity, 99.4% specificity) showing similar results with LLGP extract against the same NCC sera. The GST-T24H antigen worked better than the others in ELISA and Luminex protocols (88.3% and 96.1% sensitivity).

DISCUSSION/CONCLUSION. The WB results were very close to those presented by Noh et al. (2014) using a similar recombinant antigen (rT24H), proving that recombinant antigens can be a good alternative to crude extracts for the application in different diagnostic techniques. Furthermore, these antigens can be applied to point of care tests which would be useful in NCC field studies.
Costs involved with implementation of different post-mortem detection techniques for bovine cysticercosis in Belgium.

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A three-year study on post-mortem detection techniques for bovine cysticercosis (bcc) in Belgium delivered results necessary to model the actual current situation of bcc and taeniasis in Belgium. Prevalence of bcc appears to be much higher (36%) than assumed according to official meat inspection results (0.3%). The possible impact of implementing other post-mortem detection techniques in slaughterhouses on the occurrence of bcc and taeniasis on short and long term (10 years) was determined.

The developed model was adapted to include calculations of the costs involved with bcc and human taeniasis. Data were collected for human taeniasis patients, cattle owners and cattle insurance companies to estimate the current costs for these groups on a yearly basis. Costs for taeniasis patients include doctor visits, medication, transport to doctor and pharmacy and laboratory tests. In total, these expenses correspond to approximately 150,000 euro yearly. The Belgian health care compensates for most of the doctor visit costs, for an additional 115,000 euro yearly, for taeniasis patients only.

Cattle owners can insure their animals against bcc in 2 ways (type 1 and 2). In total, Belgian cattle is currently insured for 1,725,000 euro with the type 1 insurance and for 8,750,000 euro with the type 2 insurance. Carcasses found positive at meat inspection (MI), will be frozen to kill the cysticerci, leading to a 30-45% value loss of the meat. For cattle owners with uninsured animals, this accounts for 25,000-37,000 euro and for the type 1 and type 2 insurance companies, who are responsible for the insured animals, 125,000-185,000 euro and 346,035 – 519,053 euro, respectively can be lost due to condemnation.

Replacing the post-mortem detection technique used in slaughterhouses by more optimal detection methods will evidently change the cost related to bcc and taeniasis. The model created is capable of comparing the impact of these improved techniques on the costs for human patients, cattle owners and the insurance company on short and long term. Data are still being collected on costs concerning the MI itself (time spent, personnel cost) and implementation of Ag-ELISA (cost for test, personnel, transport to lab, etc.).
Teniosis and some other foodborne parasites in Hungary regarding 10 years period

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Introduction: Taeniosis caused by Taenia solium or Taenia saginata is for a long time known disease in Hungary, as a majority of foodborne parasites also. Some other foodborne parasites occur in Hungary are also presented.

Material, methods, results: In NCE and together with County and Regional Parasitological laboratories diagnostic stool and defecated worm suspected samples of 126 947 persons. T.saginata was diagnosed in 12 (0,01%), Taenia sp. 19 (0,02%), T.solium was not diagnosed in neither of samples. In serum/CSL of 221 persons serological examination for human cysticercosis have been performed. Human cysticercosis was not confirmed.

Regarding other FBP in diagnostic stool and defecated worm suspected samples of 126 947 persons Giardia intestinalis 1530 (1,2%), Cryptosporidium sp. 37 (0,03%), Entamoeba histolytica 118 (0,09%), Ascaris lumbricoides 242 (0,19%), Trichuris trichiura 133 (0,1%) have been diagnosed.

In serum of 102 205 persons Toxoplasma serological examination have been performed and 3 703 (3,62%) were positive. In serum of 16 924 persons Toxocara serological examination have been performed and 3 875 (22,9%) were positive. In serum of 9 217 persons Echinococcus serological examination have been performed and for E. granulosus 114 (1,24%), E. multilocularis 5 (0,005%), Echinococcus sp. 126 (1,37%) were positive. In serum of 1 557 persons Trichinella serological examination have been performed and 9 (0,58%) were positive.

Conclusion: Taeniosis, particularly T.solium/cysticercosis nowadays is rather rare in Hungary. Occurrence of some other FBP in Hungary is sporadic and correlate with occurrence in other European countries.
Seroprevalence of porcine cysticercosis in Northwestern Romania

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Introduction. Porcine cysticercosis is an infection of pigs produced by *Cysticercus cellulosae*, the larval stage of *Taenia solium*, a cestode that causes taeniosis in humans. In Romania, it is necessary to collect recent data on the prevalence of porcine cysticercosis, to detect areas of zoonotic risk, in order to prevent economic losses through proper implementation of control programs.

Aim. The present study was conducted to determine the seroprevalence of porcine cysticercosis.

Material and method. The study included four counties: Bihor, Bistrita-Nasaud, Cluj, Mures. Between July–December 2014, a total of 376 pigs (rural communities) were tested serologically. The serodiagnostic efficacy of ELISA has been studied using a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (Ag-ELISA).

Results. Only 24 out of 376 porcine blood samples were positive for cysticercal antigens. The overall prevalence of porcine cysticercosis was 6.38% (4.25%–11.7%) by Ag-ELISA. None of the seropositive animals was detected by meat inspection. Therefore, there is the possibility that the estimated prevalence is not accurate because of the misdiagnosed or undiagnosed infections, for instance due to cross reactions with *T. hydatigena* or other parasites.

Conclusions. The results demonstrate that further epidemiological studies regarding *Taenia solium* larvae infestation in porcine are required in this region of Romania. These studies must be focused on rural communities, where control measures are not rigorously applied, to properly evaluate the scale of the phenomenon. Serological surveillance diagnostic tests are mandatory to be introduced, besides the slaughterhouse basic exams. More researches are necessary to assess their real potential.
Porcine Cysticercosis in Serbia

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**Background:** The earliest official record regarding porcine cysticercosis in Serbia is a decree from 1863 which prohibits the sale of infected meat. Postmortem inspection of the animal consisted of palpation of the tongue along with visual inspection of different cuts of meat. At that time the prevalence of infection in pigs was estimated at 2% - 4%. Today, postmortem inspection includes visual examination of *psoas major*, diaphragm, heart, and other visible muscles.

**Material:** Official reports and literature data on porcine infection with *Taenia solium* in the last 40 years from Serbia were analyzed.

**Results:** Studies in the seventies confirmed the presence of cysticercosis in pigs slaughtered in slaughterhouses in Northern and Central Serbia, with a prevalence of 0.01% and 0.023%, respectively. In the last years of the former Yugoslavia (1985-1989), one study showed detection of *T. solium* in 274 out of 3,202,772 pig carcasses (0.008%). During the nineties (1994-2000), of a total of 75,823 pigs slaughtered in South Serbia, 11 infected pigs were registered (0.002%). Importantly, eight of these were detected after 1996, and although OIE provides official reports as of 1996, none of these eight were registered in the official OIE reports. There has been no survey focused on the infection of pigs after 2000. According to the official records of the Ministry of Agriculture and Environmental Protection, infection was reported in only one animal in 2004, and no cases were reported since.

**Discussion:** Official data should be taken with care, as the number of reported cases is likely to be an underestimate. Traditional home slaughtering is dominant in Serbia, and not always reported to the veterinary services. In addition, structural and economical reforms during transition have led to the replacement of large meat industrial companies that had in-house inspection with a large number of smaller abattoirs, too small for full-time veterinarian in-house inspection.
Mapping areas contaminated by Taenia solium eggs: a spatial multicriteria evaluation approach in Madagascar

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Taenia solium cysticercosis/taeniasis is endemic in Madagascar and constitutes a heavy burden for human and pig [1]. Risk mapping contributes to disease surveillance and control systems, but is a very challenging task in the absence of large epidemiological datasets [2]. We used Spatial Multicriteria Evaluation (MCE) to produce risk maps for porcine cysticercosis and taeniasis. This method transforms and combines geographical data and value judgments to obtain appropriate and useful information for decision making [3].

Six expert-based maps were created to display at-risk areas regarding the various steps of the T. solium cycle, i.e. (1) potential excretion of T. solium eggs in environment, (2) dissemination of eggs in environment, (3) potential eggs’ survival in environment, (4) potential transmission to pigs, (5) human contamination by ingestion of pork with T. solium cysts, and (6) human contamination by ingestion of food or water contaminated by T. solium eggs or by lack of hygiene. Risk factors were identified from expert-knowledge and bibliographic review. As a preliminary assessment, a common set of 25 risk factors was selected, and the corresponding geographic data collected from different data sources and institutions. They were related to soils and climate, land use, human and animal population and health, socio-economy and education. All data were integrated into a Geographic Information System (QGIS freeware, QGIS 9.12.0-Lyon, http://qgis.org/), and processed into standardized risk factor layers using spatial analysis tools. Weighted Linear Combination (WLC) was used to combine the standardized risk factor layers. Resulting maps were compared to surveillance data, i.e. contaminated carcasses reported in Antananarivo slaughterhouses, 2013-2014 [4] to assess the consistency of risk maps at regional level.

This work is the first to produce a risk assessment map at national level. Because of limited data availability, proxies of several risk factors had to be established. Some simplifying assumptions also had to be made regarding risk factor weighting. Additional data for validation (outbreak locations, case reports) need to be collected to assess the quality of the different risk maps. Finally, similar MCE approach may be implemented at local level to provide risk maps for porcine cysticercosis and taeniasis with higher spatial accuracy.

Keywords: Taenia solium; Madagascar; parasite; environment; risk mapping
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References


Visit the Pasteur Museum, a place of remembrance that first opened to the public at the Institut Pasteur in 1936. The Museum harbors the memory of the life and work of Louis Pasteur in the spacious apartment he occupied during the final seven years of his life.
Molecular characterization of *Taenia saginata* cysts in Germany

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As the beef tapeworm *Taenia saginata* causes cysticercosis in cattle and taeniosis in human this parasite is of medical and veterinary importance in an equal measure. Understanding the genetic population structure of the parasite helps to analyze parasite transmission patterns and aids in the development of control measures. A common method to clarify phylogenetic relationship among taeniid cestodes is the analysis of the cytochrome *c* oxidase subunit 1 (*cox1*) gene of mitochondrial DNA. Amongst *T. saginata* and the closely related species *T. asiatica* this gene shows high degree of conservation, whereas the NADH dehydrogenase subunit 5 (*nad5*) is the most variable mitochondrial gene.

The aim of the study was to examine the intraspecific variation and population genetics of *T. saginata* cysts from German cattle by sequence comparison of the *cox1* and *nad5* genes. Furthermore, obtained gene sequences were analyzed in relation to *T. saginata* isolates of worldwide origin.

*T. saginata* cysts were collected from cattle slaughtered at northern Germany. Cysts or cysts containing tissue were collected during routine meat inspection procedures. Moreover, *T. saginata* DNA as well as parasitic material sent by COST CYSTINET partners from Switzerland, Belgium and Palestine were included into the analyses. A total of 128 cysts and one proglottid were subjected to DNA extraction. Amplification and subsequent Sanger sequencing of *cox1* gene was successful in 57 samples, whereas 32 sequences were obtained for the *nad5* gene. Multiple sequence alignment (MUSCLE) and phylogenetic as well as molecular evolutionary analyses were conducted with the MEGA software tool. Phylogenetic classification was achieved using the neighbor-joining method. The Bootstrap consensus trees inferred from 1000 replicates. Genetic diversity values, including polymorphic sites and number of mutations amongst sequences, haplotype numbers, haplotype diversity, nucleotide diversity and theta-w as well as theta-pi as estimators to measure DNA polymorphism, were calculated using DnaSP software. The genetic structure of the parasite was elucidated under the population expansion effect via Tajima’s D test and Fu’s Fₜ test.

Sequence comparison revealed two polymorphic sites and mutations for German isolates in both genes. Moreover, three haplotypes with haplotype diversity of 0.088 for *cox1* and 0.186 for *nad5* gene as well as nucleotide diversities of 0.00028 and 0.00095, respectively, were observed.