EURO-FBP Working Group and Management Committee Meeting 2016
&
EURO-FBP WG2 Meeting “Analytical methods for foodborne parasites in human and veterinary diagnostics and in food matrices”
&
2nd CYSTINET Working Group and Management Committee Meeting 2016
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Joint EURO-FBP and CYSTINET Meeting

PROGRAMME AND ABSTRACT BOOK

26-28 September 2016
Faculty of Medicine
Ljubljana, Slovenia
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Faculty of Medicine
Ljubljana, Slovenia
Slovenian Society for Clinical Microbiology and Hospital Infections of Slovenian Medical Association
Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana
European Cooperation in Science and Technology (COST)

Ljubljana, Slovenia 2016

EURO-FBP Working Group and Management Committee Meeting 2016 & EURO-FBP WG2 Meeting “Analytical methods for foodborne parasites in human and veterinary diagnostics and in food matrices” & 2nd CYSTINET Working Group and Management Committee Meeting 2016 & Joint EURO-FBP and CYSTINET Meeting

Programme and Abstract Book

Organising Committee: Lucy Robertson, Norway
Christian Klotz, Germany
Sarah Gabriël, Belgium
Barbara Šoba Šparl, Slovenia
Miha Skvarč, Slovenia

Edited by: Barbara Šoba Šparl, Miha Skvarč

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PROGRAMME
# Working Group Meeting

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<td>9:00-10:30</td>
<td>Plenary session <em>(Lecture room 2)</em> &lt;br&gt;9:00 Reception of participants &lt;br&gt;9:15 Welcome Miha Skvarč, Barbara Šoba Šparl (IMI) – local organisers Miroslav Petrovec – Head of the Institute of Microbiology and Immunology and the President of the Slovenian Society for Clinical Microbiology and Hospital Infections of Slovenian Medical Association &lt;br&gt;9:30 Presentation of the COST action FA1408 EURO-FBP and general progress, introduction to Year 2 WBP (Lucy Robertson) Zagreb meeting status – expected output plan (Relja Beck) STSM presentation (Ricardo Santos) Early Stage Researcher Forum (Kristoffer Tysnes) Homepage (Kristoffer Tysnes) COST rules: a quick reminder (Kristoffer Tysnes)</td>
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<tr>
<td>10:30-10:45</td>
<td>Coffee break</td>
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<tr>
<td>10:45-11:05</td>
<td>Report on Bilthoven meeting (Joke van der Giessen) <em>(Lecture room 2)</em></td>
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<td>11:05-11:25</td>
<td>Report on Berlin meeting (Rachel Chalmers) <em>(Lecture room 2)</em></td>
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<tr>
<td>11:30-12:30</td>
<td>Separate Working Group session (briefing of activities since last meeting and future plans) <em>(Lecture room 2, Lecture room 3, Seminar room 2)</em></td>
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<tr>
<td>12:30-13:30</td>
<td>Lunch</td>
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<tr>
<td>13:30-14:30</td>
<td>Separate Working Group session (cont.) <em>(Lecture room 2, Lecture room 3, Seminar room 2)</em></td>
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14:30-14:45  Coffee break

14:45-16:30  Plenary session of all working groups (WG1 – Joke van Der Giessen, WG2 – Christian Klotz, WG3 – Cédric Gérard, WG4 – (Lucy Robertson)) *(Lecture room 2)*

**Management Committee Meeting**

16:30-17:30  Management Committee Meeting (for MC members only) *(Lecture room 2)*

**Guided tour of Ljubljana**

18:15  Guided tour of Ljubljana – optional *(Dragon Bridge)*
**27 September 2016**

**EURO-FBP WG2 Meeting: Analytical methods for foodborne parasites in human and veterinary diagnostics and in food matrices (Lecture room 1)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tr>
<td>8:30-8:50</td>
<td>Introduction to WG2 Presentations</td>
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<tr>
<td>8:30</td>
<td>Christian Klotz: <em>Towards generating a map of European laboratories involved in detection and diagnosis of foodborne parasites</em> (O FBP 0-1)</td>
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<tr>
<td>8:40</td>
<td>Michal Slany: <em>Currently used methods for detection of foodborne parasites: Euro-FBP survey</em> (O FBP 0-2)</td>
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<tr>
<td>8:55-10:35</td>
<td><strong>Session 1</strong>: Protozoa – Toxoplasma (Vasile Cozma)</td>
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<tr>
<td>8:55</td>
<td>Jacek Sroka: <em>A brief overview about analytical methods for detection of Toxoplasma gondii in food and water</em> (O FBP 1-1)</td>
</tr>
<tr>
<td>9:05</td>
<td>Vasile Cozma: <em>Serological epidemiology and genotypes of Toxoplasma gondii infections in Romania</em> (O FBP 1-2)</td>
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<tr>
<td>9:15</td>
<td>Joke van der Giessen: <em>Toxoplasma gondii in the main livestock species in Europe</em> (O FBP 1-3)</td>
</tr>
<tr>
<td>9:35</td>
<td>Gereon Schares: <em>Relationship between specific antibodies in chickens and the presence or infectivity of Toxoplasma gondii cysts in meat and other edible tissues</em> (O FBP 1-4)</td>
</tr>
<tr>
<td>9:55</td>
<td>Olgica Djurković-Djaković: <em>Diagnosing Toxoplasma gondii infection: different goals in man, livestock, food and environmental matrices</em> (O FBP 1-5)</td>
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<tr>
<td>10:05</td>
<td>Miha Skvarč: <em>Diagnostic accuracy of adjusted low IgG avidity index to predict acute Toxoplasma gondii infection in the first trimester of pregnancy</em> (O FBP 1-6)</td>
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<tr>
<td>10:25</td>
<td>Aleksandra Uzelac: <em>Diagnosing Toxoplasma gondii infection in haematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) patients</em> (O FBP 1-7)</td>
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<tr>
<td>10:45-11:15</td>
<td>Coffee break and group photo (EURO-FBP &amp; CYSTINET)</td>
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<tr>
<td>11:15-12:00</td>
<td><strong>Session 2</strong>: Invited speaker presentation (Karin Troell)</td>
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</table>
11:15 Jessica Kissinger, Institute of Bioinformatics, University of Georgia, USA: The value of integrated data for both basic and epidemiological studies: EuPathDB (O FBP 2-1)

12:05-12:55 Session 3: Protozoa (Rachel Chalmers)
12:05 Karin Troell: Single cell genomics in Cryptosporidium (O FBP 3-1)
12:25 Christina Skår Saghaug: Analysis of single nucleotide variants in 29 Giardia lamblia genes responsible for metronidazole metabolism and oxidative stress management (O FBP 3-2)
12:35 Kurt Hanevik: Developing a workflow for selecting and extracting Giardia genes of interest (O FBP 3-3)
12:45 Nikol Reslova: Real-time PCR versus digital PCR in diagnostics of foodborne parasites (O FBP 3-4)

13:00-14:00 Lunch and poster presentations (EURO-FBP & CYSTINET)

14:00-15:00 Session 4: Protozoa (Simone M Cacciò)
14:00 Rachel Chalmers: Best Practices in Europe: how good are we at diagnosing gastrointestinal foodborne protozoa? (O FBP 4-1)
14:20 Stéphanie La Carbona: Simultaneous detection and characterization of Toxoplasma gondii, Cryptosporidium parvum and Giardia duodenalis in vegetables and fruits (O FBP 4-2)
14:40 Laetitia Kortbeek: Risk factors for sporadic cryptosporidiosis cases in The Netherlands, analysis of a three-year population-based case-control study, 2013-2016 (O FBP 4-3)
14:50 Panagiota Ligda: Evaluation of different protocols for the detection of Giardia and Cryptosporidium in Mediterranean mussels (Mytilus galloprovincialis) (O FBP 4-4)

15:05-16:00 Session 5: Nematoda (Ivona Mladineo)
15:05 Ivona Mladineo: Anisakis sp.: diagnosis from live to canned fish (O FBP 5-1)
15:15 Maria Angeles Gómez-Morales: Serological diagnosis of Trichinella infection in humans (O FBP 5-2)
15:25 Mirosław Różycki: Validation of artificial digestion assay for detection of Trichinella spp., based on results of Proficiency Comparison results 2007-2015 in Poland (O FBP 5-3)
Mirosław Różycki: Detection of Anisakis sp. in fish from the Baltic Sea (O FBP 5-4)
15:40 Emília Dvorožňáková: Human outbreaks of trichinellosis in Slovakia since year 1980 (O FBP 5-5)
15:50 Ewa Bilska-Zając: The results of investigations on protein profile of Trichinella isolates using MALDI-TOF MS (O FBP 5-6)

16:05-16:30 Coffee break and poster presentations (EURO-FBP & CYSTINET)
16:30-17:40  **Session 6: Cestoda & Trematoda (Carmen-Michaela Cretu)**

16:30  Carmen-Michaela Cretu: Diagnosis and management of echinococcosis – Romanian experience  (O FBP 6-1)

16:50  Laetitia Kortbeek: *Specific serology for Echinococcus multilocularis in Dutch patients* - *COST FBP 2016* (O FBP 6-2)

17:00  Mario Sviben: Echinococcus granulosus *infection in Croatia* – *epidemiology and diagnostic*  (O FBP 6-3)

17:10  Santiago Mas-Coma: *Foodborne trematodiases in Europe*  (O FBP 6-4)

17:30  Hélène Yera: *Contribution of molecular identification of Diphyllobothrium*  (O FBP 6-5)

**Dinner**

20:00  Dinner at Restaurant *Gostilna na gradu (Grajska planota 1, 1000 Ljubljana)* (EURO-FBP & CYSTINET)
27 September 2016

**Working Group Meeting**

9:00-10:00  Plenary session *(Lecture room 2)*
9:00  Reception of participants
9:15  Welcome
   Barbara Šoba Šparl (IMI) – local organiser
   Miroslav Petrovec – Head of the Institute of Microbiology and Immunology and the President of the Slovenian Society for Clinical Microbiology and Hospital Infections of Slovenian Medical Association
9:30  Presentation of the COST action TD1302 CYSTINET and general progress
   Sarah Gabriël – Action Chair
   Barbara Šoba Šparl – STSM Coordinator

10:00-10:45  Separate Working Group session (briefing of activities since last meeting) *(Lecture room 2, Lecture room 3, Seminar room 2)*

10:45-11:15  Coffee break and group photo (CYSTINET & EURO-FBP)

11:15-13:00  Separate Working Group session (cont.) *(Lecture room 2, Lecture room 3, Seminar room 2)*

13:00-14:00  Lunch and poster presentations (CYSTINET & EURO-FBP)

14:00-14:45  Plenary session of all working groups (WG1 – Brecht Devleesschauwer, WG2 – Teresa Garate & Pierre Dorny, WG3 – Chiara Trevisan) *(Lecture room 2)*

14:45-16:00  Oral presentations (Sarah Gabriël) *(Lecture room 2)*
14:45  Anamaria Cozma-Petruţ: *Experimental model of chemoprevention in hepatoperitoneal cysticercosis in pigs* (O CN 1)
15:00  Emma Hobbs: *Assessment of the computer-based Taenia solium educational program ‘The Vicious Worm’ on knowledge uptake in primary school children in Katete district in Eastern Zambia* (O CN 2)
15:15  Fabian Dupont: *Risk factor analysis in patients with neurocysticercosis associated epilepsy in northern Uganda* (O CN 3)
15:30  Celine Kaae Laforet: *Fact sheets for strategic communication to the general public on Taenia solium and Taenia saginata* (O CN 4)

15:45  Chiara Trevisan: *Disease behaviours of sows naturally infected with Taenia solium in Tanzania* (O CN 5)

16:05-16:30  Coffee break and poster presentations (CYSTINET & EURO-FBP)

**Management Committee Meeting**

16:30-18:00  Management Committee Meeting (for MC members only) (*Lecture room 2*)

**Dinner**

20:00  Dinner at Restaurant *Gostilna na gradu (Grajska planota 1, 1000 Ljubljana)* (CYSTINET & EURO-FBP)
Joint EURO-FBP & CYSTINET Meeting

9:00-10:35 Plenary session: Invited speakers presentations (Karin Troell, Sarah Gabriël) (Lecture room 2)
   9:00 Isra Cruz, Senior Scientific Officer, Neglected Tropical Diseases Programme, Foundation for Innovative New Diagnostics (FIND): Diagnostics development pipeline (O FBP&CN 1)
   9:50 Jessica Kissinger, Institute of Bioinformatics, University of Georgia, USA: What types of information can be gleaned from both whole genome and targeted amplicon NGS data of pathogens? (O FBP&CN 2)

10:40-11:10 Coffee break and poster presentations

11:10-12:05 Oral presentations (Christian Klotz) (Lecture room 2)
   11:10 Joke van der Giessen: Ranking food-borne parasites in Europe using multicriteria decision analyses (O FBP&CN 3)
   11:30 Hélène Yera: Contribution of PCR in cerebrospinal fluid samples for the diagnosis of neurocysticercosis in patients living in a non-endemic country (O FBP&CN 4)
   11:40 András József Laki: Microfluidic devices for parasitology (O FBP&CN 5)
   11:50 Teresa Gárate: Evaluation of 2B2t recombinant antigen in western blot for diagnosis of cystic echinococcosis (O FBP&CN 6)

12:10-13:10 Open discussion: How to envisage (or not) the development/optimisation of tools detecting multiple pathogens in the same matrix? (Lecture room 2, Lecture room 3) (Two discussion groups, followed by a plenary wrap-up session with all participants)

   - Discussion Group 1 (Joke van der Giessen) – Human and veterinary samples: Blood (whole blood, serum), stool
   - Discussion Group 2 (Pierre Dorny) – Food: Meat (meat, meat juice), vegetables, others
Points to address within each group (considering FBP):
- Is there a need to look into multipathogen detecting tools? (pros and cons)
- Which are the multipathogen detecting tools currently existing?
- Which pathogens could be combined?
- Which techniques could be envisaged?
- Should the focus be on high throughput systems? For which regions (resource rich vs resource poor regions)?
- Do we also need tools to genotype FBP and when is this important?
- Is WGS feasible to identify FBP knowing that this is the future for bacteria and viruses?
- How can we, as a group, start/continue this?

13:10-14:10	Lunch and poster presentations

14:10-15:15	Open discussion: How to envisage (or not) the development/optimisation of tools detecting multiple pathogens in the same matrix? (cont.) (Lecture room 2, Lecture room 3)

15:15-16:00	Plenary Wrap-up session (Lucy Robertson, Sarah Gabriël) (Lecture room 2)

16:00	End of the Meeting
ABSTRACTS
Towards generating a map of European laboratories involved in detection and diagnosis of foodborne parasites

C. Klotz

Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Robert Koch-Institute, Berlin, Germany
Corresponding author: Christian Klotz, KlotzC@rki.de

One focus of Working group 2 in the COST Action European network for foodborne parasites (Euro-FBP) as stated in the Memorandum of Understanding is “Investigation of European labs with competence in analysing food matrices for specific FBP and for diagnosing infection of FBP in humans and will including mapping of European labs with such competence on a country-by-country basis.” One important sub-task is therefore to identify regional distribution of labs with specific competence in analysing different food matrices for FBPs and in diagnosing relevant FBPs in humans and animals.

In order to achieve these aims, the following survey has been conducted: All management committee members from each participant country within Euro-FBP have been contacted by email and asked to provide the information on labs with specific competence, as described, from their particular country. The information was collected using a pre-designed spread sheet. In summary, 26 of the 30 COST participant countries replied and provided the relevant information. Information from over 110 institutions with more than 130 laboratories was provided. Of these, 45 labs are involved in ‘food surveillance’, 69 in ‘veterinary surveillance’ and 67 in ‘human disease surveillance’. A first overview of the collected data will be given and further steps will be discussed.
Currently used methods for detection of foodborne parasites: Euro-FBP survey
M. Slaný

Veterinary Research Institute, Brno, Czech Republic
Corresponding author: Michal Slaný, slany@vri.cz

There exists variety of methods applied for detection of foodborne parasites. This presentation will summarize results of Euro-FBP survey focused for currently used detection methods (protozoa, nematoda, cestoda) in European laboratories.
O FBP 1-1
A brief overview about analytical methods for detection of Toxoplasma gondii in food and water
J. Sroka¹, J. Karamon¹, A. Wójcik-Fatla², V. Zając², E. Bilska-Zając¹, T. Cencek¹

¹Department of Parasitology, National Veterinary Research Institute, Pulawy, Poland
²Department of Biological Health Hazards and Parasitology, Institute of Rural Health, Lublin, Poland
Corresponding author: Jacek Sroka, jacek.sroka@piwet.pulawy.pl

Monitoring of Toxoplasma gondii in foodstuff (including water) should provide important information to risk assessment for infection of T. gondii and in consequence, for consumer protection. Therefore, the efficacy of analytical methods using to identify T. gondii in food is important for public health care. These methods need to be well characterized in aspect of their sensitivity, specificity and potential cross-reactivity with other organisms, by using reference materials and reagents. Due to the specificity of epidemiology of toxoplasmosis, meat (and meat products), goat milk, unwashed vegetables and fruits, and drinking water are as possible diagnostic matrices. Detection of parasite can be perform by several methods, including direct isolation live parasite by bioassay or tissue cell cultures, or by detection nucleic acids using molecular methods. In examination of foodstuff samples, rarely parasite can be identified only by histologic methods. PCR (conventional, nested and real-time) is the most often technique used for T. gondii DNA detection. Several multicopy targeting genes are usually using for the detection of T. gondii in samples, including B1 gene, 529 bp repeat element, internal transcribed spacer (ITS-1) or 18S rDNA sequences. Recently, the loop-mediated isothermal amplification (LAMP) targeting the T. gondii SAG1, 529 bp repetitive element, B1, SAG2, GRA1, oocyst wall protein (OWP) genes, and 18S rRNA has been developed and successfully used in the tissue and environmental samples. To identify genotypes of parasite multilocus sequence typing (MLST), PCR-RFLP, RAPD-PCR, high-resolution melting analysis (HRM) and microsatellite analysis have been also developed. Furthermore, as alternative to PCR, serotyping methods based on polymorphic polypeptides derived from T. gondii antigens SAG2, GRA3 and GRA5-7 can be used for recognize the T. gondii clonal types. Moreover, for the assessment the viability of parasites infecting sample, techniques targeting T. gondii RNA have also been developed. Recently, a sequence-specific magnetic capture method for the isolation of T. gondii DNA from large samples of tissue (100 g) combined with real time PCR was described. This method can overcome the difficulties linked with heterogeneous distribution of T. gondii tissue cysts, and potential absence of parasites in case of small sample size. There are no standardized methods for T. gondii detection in water. Usually, oocysts were concentrated from large volume of water by filtration or centrifugation, isolated by sucrose flotation or immunomagnetic separation (IMS) and detected by microscopy, PCR or bioassay. For T. gondii oocysts there are no commercially available IMS and immunofluorescent techniques. Standardized techniques, so as bioassay and tissue culture, despite on their delivering of information about viability of detected parasites, there are time-consuming and not suitable i.e. for slaughterhouse testing or monitoring of commercial meat products. Hence, alternative methods for the routine detection of Toxoplasma in meat, meat products and water (can to determine the viability of parasites) should be developed, validated and standardized.
Serological epidemiology and genotypes of *Toxoplasma gondii* infections in Romania

V. Cozma¹,², A. Gyorke¹, D. Barburaș¹, A. Cozma-Petriț³, C. Gherman¹

¹Universitatea de Științe Agricole și Medicină Veterinară Cluj-Napoca
²Academia de Științe Agricole și Silvice Bucureșt
³Universitatea de Medicină și Farmacie “Iuliu Hațieganu” Cluj-Napoca

Corresponding author: Vasile Cozma, cozmavasile@yahoo.com

Species of parasites from the *Toxoplasma*, *Taenia* and *Trichinella* genera represent the most important foodborne parasites in Romania. Specific meat inspection is not done for protozoa as *T. gondii*. Humans acquire infection with *T. gondii* most often by ingestion of tissue cysts with undercooked meat and the ingestion of oocysts via water. The main source of tissue cysts is considered to be pork. In Romania, most of the studies in intermediate hosts (humans and animals) and conducted during the last 15 years are limited to serological epidemiology. The results have revealed high prevalence of specific antibodies. Seroprevalence of anti-*T. gondii* antibodies was 59.5% in humans from north-western and central Romania. In animals were reported the following data: 70.4% in sheep and 50% in lambs; 55.8% in goats and 4.7-33.1% in kids; 30.5% in pigs raised in backyard system; 12.4% in sows; and 37.8-39% (MAT and ELISA) in horses.

Seroprevalence in animals only indicates prior exposure to *Toxoplasma* and does not necessarily indicate active infections with risk of transmission to humans. There is a large knowledge gap with regard to which lineages are present in production animals, as well as in game animals and humans. The limited genotyping data from Romania so far has indicated the presence of only type II isolate in a human case and in two kids. The different strains of *Toxoplasma* can differ in their infectivity and virulence and understanding these differences, coupled with further epidemiological studies, bioassays to screen for active infections in meat and risk assessments, could reveal factors for disease transmission that could be mitigated to limit human *Toxoplasma* infections in Romania as well as to increase understanding of the transmission cycle.
**O FBP 1-3**

**Toxoplasma gondii** in the main livestock species in Europe

J. van der Giessen¹, G. Schares², R. Blaga³, M. Opsteegh⁴ on behalf of the consortium*


¹National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands
²Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
³Alfort Veterinary School, Maisons Alfort, France
⁴French Agency for Food, Environmental and Occupational Health (ANSES), France

Corresponding author: Joke van der Giessen, joke.van.der.giessen@rivm.nl

From 2013-2015, European project was carried out in eight countries to study *Toxoplasma gondii* in the main livestock species in Europe. After extensive review of the literature, experimental studies were performed in the main livestock species (cattle, small ruminants, pigs, horses and poultry) to address the main knowledge gaps considering (1) the relationship between detection of antibodies and presence of *T. gondii*, (2) the anatomical distribution of tissue cysts, and (3) on-farm risk factors for infection. In slaughterhouse studies in cattle and horses, positive mouse bioassay results were obtained both in cattle and horses, indicating a potential infection risk for consumers. However, a lack of concordance between detection of antibodies by MAT and detection of *T. gondii* in tissues by mouse bioassay or MC-PCR was demonstrated in both species. In naturally infected pigs and chickens the agreement between direct and indirect tests was considered fair to substantial. Calves, sheep, pigs, chickens and turkeys were experimentally infected with *T. gondii*. In calves, *T. gondii* was detected in various tissues, but no clear predilection sites were identified. Brain and heart were clearly identified as predilection sites in chickens and turkeys, with higher parasite loads compared to breast, thigh and drumstick muscle, although drumstick, an edible tissue, was indicated a potential risk for consumers. Laying hens with outdoor access were more often infected at small backyard farms compared to large organic farms in Germany. Vaccination with S48 strain *T. gondii* resulted in reduced tissue cyst formation in vaccination and challenge studies in sheep and pigs.


This research was conducted by a consortium within the framework of a grant agreement n° GA/EFSA/BIOHAZ/2013/01 funded by the European Food Safety Authority. This paper is published under the sole responsibility of the authors, and shall not be considered as an EFSA output.”
O FBP 1-4

Relationship between specific antibodies in chickens and the presence or infectivity of *Toxoplasma gondii* cysts in meat and other edible tissues*

G. Schares¹, B. Bangoura², M. Koethe³, M. Ludewig³, T. Goroll², F. Randau², P. Maksimov¹, B. Matzke⁵, M. Sens¹, A. Bärwald², I. Villena⁴, D. Aubert⁴, F. J. Conraths¹, M. Opsteegh⁵, J. Van Der Giessen⁵ on behalf of the European Food Safety Agency project GP/EFSA/BIOHAZ/2013/01

¹Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
²Institute of Parasitology, University Leipzig, Germany
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⁵National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

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Meat appears to be a major source of *Toxoplasma gondii* infections in Europe. To gain more insight into the role of meat as a source of human infection with *T. gondii*, it is important to have an indication on the prevalence of infectious tissue cysts in the main livestock species. Serological assays are commonly used to determine the prevalence, but the predictive value of seropositivity with respect to the presence of infective tissue cysts in various livestock species is unknown. The present study aimed at the identification of seropositive and seronegative chickens from organic and backyard farming and their relation with the presence of viable tissue cysts. 30 laying hens, which had tested seropositive in an in-house TGSAG1-ELISA, were selected from more than 400 hens sampled on these farms. Hearts and limb muscles of seropositive chickens were homogenized, treated with an acid pepsin solution and examined for *T. gondii* tissue cysts in a bioassay with gamma-interferon-knockout or gamma-interferon-receptor-knockout mice. In addition, 30 hens, which were seronegative in the TGSAG1-ELISA, were selected and examined in the same way. Viable *T. gondii* could be isolated from the majority of seropositive and from a few seronegative chickens. Most *T. gondii* isolates were obtained in the bioassay using chicken heart samples, while limb muscles were rarely positive.

*This research was conducted by a consortium within the framework of project n° GA/EFSA/BIOHAZ/2013/01 entitled “Relationship between seroprevalence in the main livestock species and presence of Toxoplasma gondii in meat”, funded by the European Food Safety Authority.*
Diagnosing *Toxoplasma gondii* infection: different goals in man, livestock, food and environmental matrices

O. Djurković-Djaković

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*Toxoplasma gondii* is positioned at the top of any list ranking foodborne parasites according to public health relevance, whether at the global, European or regional levels. This naturally increases the pressure to provide appropriate methods to control this parasite, and therefore decrease if not eradicate human infection and health consequences. After decades of understanding and treating the consequences of human infection, the focus has, justifiably, switched to prevention, which in turn led to efforts to understand and control the infection at the source, i.e. in food (tissue cysts) and in the environment (oocysts). The range of weaponry to diagnose infection includes classical parasitological (bioassay), immunological (serology) and molecular methods that are specific, sensitive and widely available. However, this may also be the birthplace of the problems. Can we expect to use the same methods to achieve different goals? It does seem logical to use the same tools in the detection of the infection in humans and animals, since the biological materials are essentially the same. However, a deeper look shows that the available methods work quite nicely in human medicine, where the goal is to a) distinguish immunized from unimmunized women prior to or during pregnancy; b) diagnose acute infection; c) diagnose congenital infection (WB, bioassay, PCR); d) diagnose infection consequences (ocular toxoplasmosis, reactivated toxoplasmosis in the immunosuppressed individual. Distinguishing unimmunized from immunized women has for decades been performed based on a cut-off titer of specific IgG antibody. But direct extrapolation of this knowledge, for instance, to detect infection in food animals with the aim to assess those that pose a risk to human health, has not proved useful. The EFSA project, and other work, have shown no direct correlation between antibody titer and detection of tissue cysts in edible tissues, and the correlation seems to get lower as the animal gets larger. Attempts to find such correlations have led to decreasing the cut-off titer particularly of the MAT as the generally used test (albeit not validated!), to a level far too low (1:6) to be considered specific, at least in diagnosis in humans. In contrast, ELISAs specific for particular animal species use much higher cut-offs, with, reportedly, better correlation between antibody and tissue cyst detection, and it just may turn out that MAT, seductive as it may be to have a one-test-fits-all, is not THE serological tool for these purposes. Similarly, while PCR has its clear and singular place in the diagnosis of human cases, generally being the more effective as the biological material in which it is used decreases in volume (ergo, its use in amniotic, ocular, cerebrospinal fluid and bronchoalveolar fluids), the relevance of occasional (not so rare) positive PCR findings in meat/meat products not associated with tissue cyst detection, has yet to be established. On the other hand, PCR has been successfully used for the detection of Toxoplasma in seafood, produce, water and soil samples but is currently impractical, technically but also logically. As it may lead to caution against the use of fresh produce, water, etc. Could we first need fresh thinking?
Diagnostic accuracy of adjusted low IgG avidity index to predict acute *Toxoplasma gondii* infection in the first trimester of pregnancy

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One hundred and twenty-six women that came to gynaecologist for *Toxoplasma gondii* screening test during pregnancy were included in the study. When the suspicion of infection in the first trimester of pregnancy was raised women were included in the follow up scheme and their serology status was suggested to be checked once per month till delivery. As a control group, women that definitely got infected in the second or third trimester of pregnancy (seronegative *T. gondii* status at first measurement of immunoglobulin’s) were compared. The study was conducted under the guidance of Slovenian National Medical Ethics Committee.

To confirm acute infection with *T. gondii* during the first trimester of pregnancy we have set the following criteria: at the first check up the IgM and IgG should be present and IgG avidity index should be low or moderate. If the concentration of IgG and IgG avidity index increased and the concentration of IgM decreased at the next follow-up 3-4 weeks later, then we assumed that the woman got infected during the first trimester of pregnancy. Serology was monitored by IgM, IgG and IgG avidity index testing on the LIAISON XL automated diagnostic system (LIAISON® Toxo IgG II, LIAISON® XL Toxo IgM, LIAISON® XL Toxo IgG Avidity, Diasorin, Italy). In cases of suspected congenital infection IgA (Platelia Toxo IgA, Bio-Rad, Germany), IgM and IgG were measured in new-born’s blood and compared to their concentration in mother’s blood. If the congenital infection could not be excluded with measurements of immunoglobulin’s western blot mother/newborn IgG and IgM profile (WB IgG-IgM profile) in serum was performed (TOXOPLASMA WB blot IgG-IgM, LDBIO, France). To detect *T. gondii* DNA in amniotic fluid we developed in house real time PCR (qPCR).

| Table 1: Baseline data and mean IgG avidity index for 126 women included in the study |
|-----------------------------------|-----------------------------------|-----------------------------------|
| Infection before pregnancy | Possible acute infection early in pregnancy | Seroconversion |
| Number of pregnant women | 58 (46%) | 39 (31%) | 29 (23%) |
| Mean weeks of gestation ± SD | 10.7 ± 3.9 | 11.2 ± 2.6 | 26.5 ± 5.5 |
| Mean IgG avidity index ± SD | 0.20 ± 0.06 | 0.09 ± 0.04 | 0.07 ± 0.04 |

SD: standard deviation. IgG: immunoglobulin G.

The difference in IgG avidity index was statistically significant when we compared positive women with IgG avidity index in the range 0-0.11 to all other ranges (P<0.0001). If IgG avidity index is above 0.11 in a woman that has IgG and IgM present than statistically significant certainty exists that she got infected before current pregnancy (OR 0.11, 95% CI 0.05-0.25, P<0.0001).
Table 2: Number of women that possibly got infected with *Toxoplasma gondii* in the first trimester of pregnancy and mean IgG avidity index adjusted to calculated cut-offs and presented as ranges

<table>
<thead>
<tr>
<th>IgG avidity range</th>
<th>Number of women</th>
<th>Mean IgG avidity index ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.11</td>
<td>37</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>0.11-0.15</td>
<td>13</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>0.15-0.2</td>
<td>16</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>&gt;0.2</td>
<td>31</td>
<td>0.25 ± 0.03</td>
</tr>
</tbody>
</table>

SD: standard deviation, IgG: immunoglobulin G.
Diagnosing *Toxoplasma gondii* infection in haematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) patients

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The primary route of transmission of the *Toxoplasma gondii* parasite between intermediate hosts in many parts of the developed world is via ingestion of parasite laden tissue cysts from food. In the European population, *T. gondii* infection is nearly always foodborne and in rare instances waterborne. Even cases considered to be special, due to parasite transmission from mother to foetus, or via transplants or blood transfusions, the presence of the parasite in the mother or the donor material ultimately can be attributed to foodborne transmission. *T. gondii* infection is predominantly and primarily diagnosed in humans by detection of specific antibodies, despite the fact that a plethora of PCR protocols for the detection of several specific *T. gondii* DNA sequences have been developed and are in widespread use. Detection of the *T. gondii* 529 bp repeat element sequence by PCR, which combines high specificity with sensitivity (detection limit of 1 parasite/ml of blood) stands out as highly useful in diagnostics. However, even the best PCR protocols cannot overcome the limit imposed by the fact that the presence of parasites (and/or their DNA) in human peripheral blood, which is the dominant sample type available for diagnostic examination, is rare and usually short lived. Therefore, PCR is often only a supplementary diagnostic method for peripheral blood, and practically unnecessary for acute infection. However, in HSCT and SOT patients, PCR based detection of *T. gondii* DNA stands alone as the most reliable diagnostic method, as specific antibody detection may be altered by immunosuppression and/or transfusions. Several years of experience with PCR based pre-transplant and post-transplant monitoring of HSCT and SOT patients for *T. gondii* infection in our laboratory have shown the diagnostic significance of PCR-based diagnosis in peripheral blood samples in particular from HSCT patients, but also confronted us with unique technical challenges which may greatly affect data interpretation.
We are at an interesting juncture in human health and parasite studies in that research fields that have historically been separated, epidemiology/surveillance efforts and Host-Pathogen basic biology are intersecting as a result of genome and other Omic technologies. This talk will introduce the community to a well-established integrated database system in which the tools are built to facilitate research on each side of the fence as well as permit queries across the data from the different fields. The eukaryotic pathogens database, (EuPathDB) family of databases include resources for: Amoeba, Babesia, Cryptosporidium, fungi, Giardia, Leishmania, Microsporidia, Plasmodium, Toxoplasma, Trichomonas and Trypanosomes.
Infectious disease involving multiple genetically distinct populations of pathogens is frequently concurrent but difficult to detect or describe with current routine methodology. Cryptosporidium sp. is a widespread gastrointestinal protozoan of global significance in both animals and humans. It cannot be easily maintained in culture and multiple infections have been reported. To explore the potential use of single cell genomics methodology for revealing genome-level variation in clinical samples from Cryptosporidium-infected hosts, we sorted individual oocysts for subsequent genome amplification and full-genome sequencing.

Cells were identified with fluorescent antibodies with an 80% success rate for the entire single cell genomics workflow, demonstrating that the methodology can be applied directly to purified faecal samples. Ten amplified genomes from sorted single cells were selected for genome sequencing and compared both to the original population and a reference genome in order to evaluate the accuracy and performance of the method. Single cell genome coverage was on average 81% even with a moderate sequencing effort and by combining the 10 single cell genomes, the full genome was accounted for. By a comparison to the original sample, biological variation could be distinguished and separated from noise introduced in the amplification. As a proof of principle, we have demonstrated the power of applying single cell genomics to dissect infectious disease caused by closely related parasite species or subtypes. The workflow can easily be expanded and adapted to target other protozoans, and potential applications include mapping genome-encoded traits, virulence, pathogenicity, host specificity and resistance at the level of cells as truly meaningful biological units.
Analysis of single nucleotide variants in 29 *Giardia lamblia* genes responsible for metronidazole metabolism and oxidative stress management

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\textbf{Background:} The number of treatment refractory *Giardia* is increasing, and has appeared with prevalence up to 20%. The redox system in *Giardia* is a target for metronidazole (MTZ). Some proposed mechanisms for resistance include a decrease of 2-oxoacid reductase activity, including pyruvate-ferredoxin oxidoferredoxin and ferredoxin I, rearrangements of chromosomes and genotypic variations. MTZ metabolism genes are therefore of particular interest for initial assessment of markers and mechanisms of resistance in *Giardia* and can be investigate by whole genome sequencing (WGS).

\textbf{Aim:} The main aim of this study was to analyse the variability by frequencies of single nucleotide variants in a set of 29 genes in MTZ metabolism and oxidative stress management pathways to inform further work to identify genetic markers of resistance.

\textbf{Methods:} 11 clinical isolates of *Giardia* assemblage A2 and 8 assemblage B trophozoites were cultured at the Robert-Koch institute. DNA from the trophozoites was extracted using: Maxwell® 16 FFPE Plus LEV DNA Purification Kit. The quality and amount of DNA was validated with NanoDrop and Qubit. The DNA was thereafter prepared and indexed using Illumina TruSeq DNA PCR-Free Sample Prep Kit. Then validation, normalization and pooling of a DNA library with KAPA Library Quantification Kit for Illumina sequencing platforms & Agilent Technologies 2100 BioAnalyzer, High Sensitivity DNA chip was done. Sequencing was carried out using the Illumina MiSeq paired-end technology (2 x 300 bp). To be able to analyse the data, the reads from the WGS was aligned to the corresponding reference genomes of *Giardia* assemblage A and B using Bowtie2. Variant calling was then done using SamTools 1.2 and filtered with VCF-miner to explore nucleotide differences compared with the assemblage reference genomes.

\textbf{Preliminary results:} The number of nucleotides different from the reference genomes was found by dividing the variants by the total number of nucleotides. The percentage of variants in *Giardia* assemblage A and B was 0.9% and 4.5%, respectively. Overall, 489 variants in 8 A2 isolates and 2809 variants in assemblage 11 B isolates were found in the 29 genes examined. To be able to compare the number of variants within these genes, the number of variants was divided by the length of each gene. Comparison between the two assemblages revealed that *Giardia* assemblage B had generally more variants than assemblage A2, with one gene, nitroreductase family protein (GINR2), having no variants in assemblage A. The genes presenting most variants in assemblage A were thioredoxin peroxidase (1.8%), CoA-disulfide reductase NAD(P)H (1.5%), flavohemoprotein (1.4%) and axoneme-associated
protein (1.2%). The genes with most variants in assemblage B were ferredoxin (7.1%), nitroreductase Fd-NR2 (GINR1) (4.2%), thioredoxin peroxidase (3.8%) and thioredoxin-like protein (3.6%).

**Discussion:** The genes involved in the metabolism and redox-system in *Giardia lamblia* assemblage A and B had a large number of variants, ranging from 1.2-7.1% in the four genes with most variants in each assemblage. Assemblage B had more variants than A. Analyses of synonymous and non-synonymous variants are underway and will be presented.
Developing a workflow for selecting and extracting *Giardia* genes of interest
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Background: With more *Giardia* isolates being sequenced, effective methods for analysis and comparisons are necessary. There are many software alternatives, available for these analyses, many of them requiring programming or Unix skills. There is a need to simplify tools and make these analyses more efficient and accessible to more researchers and laboratory personnel without detailed bioinformatics competence. A common question is how phylogenetically close a specific isolate is to other isolates.

Aim: To set up a workflow to select and align genes of interest from mapped reads in user friendly windows based program to determine phylogenetic relationships.

Method: 20 cultured isolates originating from *Giardia* infected humans were sequenced using the Illumina MiSeq paired-end technology (2 x 300 bp), and aligned to version 26 of the corresponding *Giardia* DB reference strains DH_A2 or GS_B using Bowtie2. Bam-files were imported into the commercially available software Genious R9 (Biomatters Ltd, New Zealand) and aligned again to their reference genome. An automated workflow within this program was developed to select specified contigs with genes of interest, make a consensus of the reads mapped to this contig, extract genes of interest from the relevant consensuses, and sort these genes by name in list. Further, the workflow can concatenate the specified genes into one sequence, and an alignment and/or phylogenetic tree be built.

Result: The workflow was employed to 20 sequenced and mapped *Giardia* isolates and the concatenated sequences were aligned successfully. A phylogenetic tree showing the placement of each isolate was produced which showed a clear distinction between the A2 and B assemblage, as well as differences within the subgroups.

Conclusion: Establishing a workflow in Geneious is a simple and efficient and way to select and extract consensus sequences of specific genes from sequenced *Giardia* isolates for to evaluate phylogenetic distance and further comparisons of genes between isolates.
Real-time PCR versus digital PCR in diagnostics of foodborne parasites
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In recent years, an increased number of reported cases of human infections by parasitic agents present in foodstuffs is recorded in Europe. Among the dominant pathogens are documented protozoa - *Giardia intestinalis* and *Toxoplasma gondii*. Cysts of *G. intestinalis* can survive long term in a cool and moist environment and contaminate e.g. fruit and vegetables growing in soil. Whereas *T. gondii* is an obligate intracellular parasite of warm-blooded animals, which forms tissue cysts in nervous and muscle tissue of the intermediate host; during the meat processing might be contaminated also tissue of uninfected hosts. Moreover, meat inspection of *T. gondii* at the slaughterhouses is not legislatively established.

The detection possibilities of *G. intestinalis* and *T. gondii* were tested using two molecular methods - quantitative real-time PCR (qPCR) and digital PCR (dPCR). While qPCR is a commonly used method for the detection of foodborne parasites, dPCR represents a new potentially fast, accurate and sensitive platform on this field. dPCR DNA chips enable to screen the absolute number of specifically amplified DNA molecules, which means that the high detection limit of pathogens could be reach.

The aim of our study is to compare the efficiency of two methodological approaches adopted for detection of parasites in artificially contaminated samples of meat and vegetables by defined amounts of *G. intestinalis* (trophozoites) and *T. gondii* (oocysts).

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Laboratory diagnosis is required to identify infections with Cryptosporidium spp., Giardia duodenalis and Cyclospora cayetanensis, thus enabling the investigation and management of cases, provision of advice on the prevention of spread, and to inform surveillance to identify clusters or outbreaks. The diagnostic methods differ. For Giardia the usual diagnostic methods are either bright-field microscopy of formol-ether concentrated stools or antigen detection assays. For Cyclospora, microscopy can be augmented by examination under ultraviolet light, but staining faecal smears by the modified Ziehl-Neelsen (mZN) method provides a permanent record and is the easiest method of diagnosis. There are no antigen detection assays for Cyclospora. For Cryptosporidium stained microscopy (mZN, auramine phenol and fluorescent antibody stains) or antigen detection assays are used. The methods differ in their diagnostic sensitivity and specificity for each parasite. We have compared methods for Cryptosporidium and shown that, during acute clinical episodes, auramine phenol stained microscopy and enzyme immunoassays provide improved diagnostic sensitivity and specificity compared to mZN and immunochromatographic lateral flow assays, but the best methods are immunofluorescent antibody tests and PCR (Chalmers et al., JMM 2011; 60: 1598–1604). By sampling patients daily throughout their infection and after symptoms ceased we have establish shedding and detection profiles using different detection methods. For recuperating patients, PCR of DNA extracted directly from stools had the highest diagnostic index (Chalmers et al., submitted). The use of molecular methods for the diagnosis of gastroenteritis is increasing and Cryptosporidium spp. (especially C. parvum and C. hominis) and G. duodenalis are included in parasitology panels, but C. cayetanensis is rarely included. This means that robust algorithms for testing and reporting and maintenance of parasitological competence are needed. Diagnostic laboratory performance measured by the results of participation in the UKNEQAS Faecal Parasitology scheme is consistently excellent for Cryptosporidium (around 95% of participants achieve the correct result) which contrasts with disappointing results for C. cayetanensis (34% and 51% of participants achieving the correct result in two recent distributions). C. cayetanensis has been sent out to labs since 2003. One problem was misidentification of Cryptosporidium for Cyclospora and vice versa, indicating that laboratories were not using an eyepiece graticule to measure oocysts. Testing policies and practices vary between laboratories: some laboratories will only test on specific request from clinicians. The results of surveys in the UK will be presented (Chalmers et al., JMM 2015; 64: 688-69; Lui et al., in preparation). Furthermore, reporting of results for public health purposes and disease surveillance differs: in the UK the diagnosis of Cryptosporidium and Giardia are notifiable but Cyclospora is not. The consequences of this will be presented.

This presentation will focus on recent findings from experimental work and lessons learnt from recent outbreaks and incidents which should lead to improvements in diagnosis and reporting of foodborne parasites.
Simultaneous detection and characterization of *Toxoplasma gondii*, *Cryptosporidium parvum* and *Giardia duodenalis* in vegetables and fruits

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*Toxoplasma gondii*, *Cryptosporidium* spp. and *Giardia duodenalis* are emerging pathogen parasites in the food domain. However, without standardized methods for their detection in food matrices, parasitic foodborne outbreaks remain neglected. In this study (Hohweyer *et al.*, Food Microbiol 2016; 57: 36–34) a new immunomagnetic separation assay (IMS Toxo) targeting the oocyst’s wall of *T. gondii* was developed using a specific purified monoclonal antibody. Performances of this IMS Toxo coupled to microscopic and qPCR analyses were evaluated in terms of limit of detection (LOD) and recovery rate (RR). On simple matrix (PBS buffer), mean RR of 46±15% and 11±8% were obtained by microscopy and qPCR respectively. For both detection methods, the LOD was set at 5 oocysts/sample. When applied to food matrices, IMS Toxo coupled to qPCR analyses led to mean RR of 35 and 29% on basil and raspberries respectively. By microscopic analyses, mean RR dropped to ≤ 2%. Whatever the detection method, a LOD of 33 oocysts/g was reached. In order to increase the sensitivity of the detection of *T. gondii* in food matrices, oocysts were directly concentrated (without IMS Toxo) from the supernatant of the IMS of *Cryptosporidium* and *Giardia* (oo)cysts. This strategy associated to qPCR detection allowed to lower the LOD below 1 oocysts/g for *T. gondii* and led to RR of 2.5 and 35% on raspberries and basil respectively. For *C. parvum* oocysts, mean RR of 13% was obtained, while RR of *G. duodenalis* cysts varied between 2% on basil and 21% on raspberries. LOD ≤ 3 (oo)cysts/g were reached on the two tested matrices for both protozoa. Although lower recovery rates are obtained using molecular methods compare to microscopic analyses, these assays display many advantages: easier to implement and standardize for routine analyses, shorter time-to-result, specific to *T. gondii*, *C. parvum* and *G. duodenalis*, and access to other crucial data such as the genotype for epidemiological studies, the pathogenicity and/or the viability of the parasites.

To characterize the viability of protozoa detected on food matrices, RT-qPCR assays were developed (Travaillé *et al.*, Food Control 2016; 59: 359–365). mRNA of the three protozoa were extracted and purified directly from the pellet of matrix, and then viable protozoa were specifically detected by RT-qPCR. This procedure led to RR varying between 20 to 35% depending on the parasite and to LOD of 3 (oo)cysts/g on basil, while no mRNA could be detected on raspberries.

Overall, these data suggest that each food matrix displays specific characteristics that may interfere with protozoa extraction/elution (trapping, adhesion force) and qPCR detection (inhibitors). Moreover the
structure and intrinsic properties of each protozoa should be considered in order to determine the optimal conditions for extraction of (oo)cysts and their DNA for qPCR analyses. Hence, the development of a unique common strategy for the three protozoa and all types of food matrices is a real challenge, in order to be able to propose routine analyses of food samples at a reasonable cost.
Risk factors for sporadic cryptosporidiosis cases in The Netherlands, analysis of a three-year population-based case-control study, 2013-2016
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Background: In 2012, cryptosporidiosis cases increased in the Netherlands, but no single source was identified. The predominant species was Cryptosporidium hominis (88%). In 2013, we began a three-year population-based case-control study to identify risk factors for sporadic cryptosporidiosis in collaboration with 17 Dutch microbiology laboratories.

Methods: We defined cryptosporidiosis cases as laboratory confirmation via microscopy or PCR. Cryptosporidium samples were further analysed by RT-PCR for C. hominis or C. parvum speciation.

Cases received the study questionnaire from their GP. Controls were selected from the population register and frequency matched on age. We calculated adjusted odds ratios (aOR) using logistic regression.

Results: Overall, 650 Cryptosporidium samples were speciated. In study-year-one, 240 samples were C. parvum (81%) and 55 C. hominis (19%); study-year-two, 182 samples were C. parvum (90%) and 21 C. hominis (10%), and study-year-three, 65 samples were C. parvum (43%) and 87 C. hominis (57%). In risk factor analysis, cases in study-year-one were more likely than controls to swim in a river or lake (aOR 4.9; 95% CI: 2.0-12), have contact with cattle (aOR 3.8; 95% CI: 2.0-7), eat barbequed foods (aOR 3.8; 95% CI: 2.4-6.1) and have a household diarrhoeal case (aOR 1.8; 95% CI: 1.1-3.7). In study-year-two, cases were more likely than controls to have contact with a non-household diarrhoeal case (aOR 3.0; 95% CI: 1.2-7) or household diarrhoeal case (aOR 2.8; 95% CI: 1.3-5.8). In study-year-three, cases were more likely than controls to have a household diarrhoeal case (aOR 2.5; 95% CI: 1.7-3.9), changed infants’ diaper(s) (aOR 1.8; 95% CI: 1.1-2.8) and bathed in an inflatable pool (aOR 1.6; 95% CI: 1.1-2.5).

Conclusion: During the three-year study, we identified a species shift from C. parvum to C. hominis. Information about Cryptosporidium exposure during outdoor recreational activities and household hand-hygiene improvements could prevent future infections.
Evaluation of different protocols for the detection of *Giardia* and *Cryptosporidium* in Mediterranean mussels (*Mytilus galloprovincialis*)

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Mussels (*Mytilus* spp.), as filter organisms, accumulate pollutants in their tissues at levels proportional to their biologically available concentration in the marine environment. Thus, they can be used as indicator organisms to estimate the presence of pathogens in a given marine environment and the risk of transmission to humans by their consumption. Although *Cryptosporidium* and *Giardia* have previously been documented from mussels in many places worldwide, there is no standard method for their detection and quantification in shellfish.

We performed a comparative study between commonly used methods to detect *Cryptosporidium* and *Giardia* in mussels. As test material, 5 mussels (*M. galloprovincialis*) were homogenized, spiked with 1000 *Giardia* cysts/g and 1000 *Cryptosporidium* oocysts/g and treated with (i) pepsin digestion solution (20ml 1M HCl, 80ml distilled water, 1g pepsin) (ii) PBS/diethyl ether (2:1) or (iii) water. Following treatment, each sample was divided in two sub-samples and further processed either by immunomagnetic separation (IMS) or not. Finally, *Cryptosporidium* and *Giardia* (oo)cysts were counted by means of an immunofluorescence assay (IFAT). In total, 6 different protocols were performed, including: a) pepsin digestion, IMS and IFAT; b) pepsin digestion and IFAT; c) diethyl ether, IMS, and IFAT; d) diethyl-ether and IFAT; e) IMS and IFAT; f) only IFAT. Each experiment was repeated four times. For each replicate, negative control, i.e. unspiked samples, were included and processed. Mean recovery rates (± stdev) for *Cryptosporidium* varied from 1.0 (± 2.2)% (IFAT only) to 61.4 (± 26.2)% (pepsin + IMS + IFAT). *Giardia* recovery rates varied from 0% (ether + IFAT) to 39.6 (±14.7)% (pepsin + IMS + IFAT). These results suggest that the most sensitive/effective protocol to detect *Giardia* and *Cryptosporidium* in *M. galloprovincialis*, is digestion of the samples using pepsin solution followed by IMS and IFAT. Further work will aim at determining recovery rates of this method at lower parasite concentrations.
Elucidating Anisakis transmission patterns between populations of definitive and intermediate/paratenic hosts is a prerequisite for substantial evaluation of the disease risk in men, moreover because current data evidence that distinct species of Anisakis elicit distinct level of pathogenesis in men. Although different molecular tools have been developed recently, the methodology for basic isolation of larvae from fish as a raw material for fishery industry is still non-uniformed, introducing over- or underestimation bias in interpretation of actual values of larvae in fish. Candling, press/UV method or artificial digestion alone or in combination are the most frequently used methods depending on the sample batch size. Molecular tools used for genetic identification of larvae from fish (allozyme electrophoresis, PCR-RFLP, cox2, ITS, microsatellites) have been now optimized for use in fish products (HMR, qPCR) but still with some constrains. It seems that development of analytical methods employed in other nematosis did not fully followed the practices used in case of Anisakis, reflecting the fact that anisakiasis is still an underestimated and misdiagnosed zoonotic disease in Europe.
The lack of pathognomonic signs or symptoms in the course of trichinellosis in humans makes the clinical diagnosis of the infection difficult. Therefore, the diagnosis should be based on the anamnesis (epidemiological data and patient history of exposure), clinical evaluation and laboratory tests including serology. For serology, the International Commission on Trichinellosis recommends the ELISA and the western blot as confirmatory test, based on excretory/secretory antigens. Both tests have been standardized and properly validated in some research institutes, yet this is not the case for serological kits present on the market, resulting in their unreliability. Main problems of commercial kits are related to the antigens used, since they are not well-defined or well-characterized products. Moreover, an acceptable serological assay has to be properly validated for its intended purpose using an appropriate panel of serum samples, including sera from persons with a confirmed diagnosis of trichinellosis, which are hardly available. Most of the commercially available kits lack of information regarding validation (e.g., sample size on which sensitivity, specificity, cross reactivity have been determined). For these reasons, there is an urgent need to harmonize assays among experienced laboratories and compere their performances with the kits present on the market by ring trials.
Trichinellosis is a widely spread parasitic zoonosis caused by consumption of meat containing live larvae of the *Trichinella* genus (Pozio, 2007). The disease presents a real public health risk. Therefore efforts have been focused on control of *Trichinella* in domestic pigs that are the main source of the disease. Among control measures the magnetic stirrer digestion method is recognized as an effective tool for preventing clinical trichinellosis in humans.

The digestion method is accepted as the international reference method, and is based on enzymatic digestion of muscle tissue in an artificial digestion fluid prepared with pepsin and hydrochloric acid. Test sensitivity is greatly influenced by the sample size, by the kind of examined muscle, and the skills of the personnel. Council Regulations (EC) No 853/2004, No 854/2004 and No 882/2004 of the European Parliament laid down hygienic rules for production of food of animal origin together with requirements regarding official controls. This lead to the specific requirements for *Trichinella* controls set up in Commission Regulation (EC) No 2075/2005 of 5 December 2005. For the purpose of the official control of *Trichinella* the magnetic stirrer method for pooled-sample digestion was recommended as a reliable method for routine use and as reference method.

According to mentioned regulation, all personnel involved in the examination of samples to detect *Trichinella* should participate in a quality control programs and a regular assessment of the testing, recording and analysis procedures used in the laboratory. Proficiency testing is an established instrument for quality assurance. It is also a proof for accreditation bodies, for competence of the laboratory. Proficiency test are widely used to improve laboratory performance. The presented current study presents the effectiveness and efficacy of proficiency testing organized by Polish National Reference Laboratory with close cooperation with European Union Reference Laboratory for Parasites. Presented data were collected in 2007-2015 in Poland. Collected data enable us to establish parameters characterizing the magnetic stirrer digestion method as so called field validation. Within last seven years veterinary laboratories have examined over 18 000 PT samples, with mean accuracy 96%, sensitivity 95% and specificity 97%. We can assume that organized tests were helpful tool to increase quality of laboratory work and good source of information for method validation.
Detection of *Anisakis* sp. in fish from the Baltic Sea

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The herring (*Clupea herrengus*) and cod (*Gadus morhua*) belong to the most commonly caught fish species in the Baltic Sea. These fishes may play a role of paratenic hosts for the parasites from *Anisakidae* family: *Anisakis simplex* and *Pseudoterranova decipiens*. The ingestion by human of *Anisakidae* parasites larvae (L3) causes disease called anisakidosis. Two clinical syndromes may appear: gastrointestinal manifestations (caused by viable larvae) or allergic reactions to antigens of these parasites. The antigens are allergenic even in case of dead larvae (after freezing, high temperature treatment or other technological process). Therefore, monitoring of the presence of both parasites and their allergens in fish are important concerning public health.

The aims of the present study were to investigate the prevalence of infection and parasite fauna composition of *Anisakidae* larvae in herring and cod sampled in Baltic Sea. Totally 246 herrings and 228 cods has been collected from three ICES subdivisions of the Baltic Sea (24, 25, 26), by the 2015 and first part of 2016. Fish samples were examined by three methods: candling, candling with UV and artificial digestion. Overall, 14.23% of herring and 9.65% of cod were found to be infected. The level of infection in herring varied from 2.35% (ICES-25) to 23% (ICES-26) and for cod from 6% (ICES-24) to 15% (ICES-25). The DNA analysis (RFLP) has been used to distinguish the species composition of found *Anisakidae* parasites.

Twenty larvae from cod were identified as *A. simplex* s.s. and two as *P. decipiens*. Larvae from herring represented species of *A. simplex* s.s. in 30 cases, and 5 larvae were classified as *P. decipiens*.

The risk of acquiring anisakiosis (caused by viable larvae) in human is usually efficiently prevented by fish freezing, however our findings highlights that the presence of *Anisakidae* larvae in herring and cod, may be the source of allergic reactions in case of sensitized persons. The increasing interest of consumers for fish and fishery products (especially raw or underdone meals like sushi, marinated fish, smoked fish etc.) may pose considerable food safety problems that merits to be systematically investigated.

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Human outbreaks of trichinellosis in Slovakia since year 1980
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Human trichinellosis was more frequent in Slovakia in the past, with pig meat as the most common source of the infection. In former Czechoslovakia, obligatory meat inspection by trichinoscopy was established in 1941. The Eastern Slovakia had been known for a long time as area with human trichinellosis occurrence, twelve human epidemics had arisen between years 1939 – 1980 in this territory. Later, six epidemics were recorded between years 1980 and 2008, two of them affected the Central and Western Slovakia. In Slovakia, the most frequent species is *Trichinella britovi*, causing disease mainly in wild life species. *T. spiralis* occurs less frequently and *T. pseudospiralis* only sporadically. The epidemiology of the last six human *Trichinella* infection outbreaks between 1980 and 2008 is described in this study. Wild boar meat was the main source of infection before the nineties (Bardejov district in 1980, Poprad/Spišská Nová Ves districts in 1992, all Eastern Slovakia). Later, risk farm practices, especially feeding of pigs with the wild animal’s offal contributed to the formation of synanthropic cycle and pig meat caused the epidemics in 1990 (Vranov nad Topľou district, Eastern Slovakia), 2001 (Komárno district, Western Slovakia) and 2008 (Rožňava district, Eastern Slovakia). Sausages prepared from pork and dog meat, coincidentally infected with *T. britovi*, and offered as a local food speciality on traditional folk festival in 1998 (Brezno district, Central Slovakia) were the source of the largest human outbreak documented in Slovakia. The anti-*Trichinella* antibodies were detected in 336 event visitors. Besides mentioned epidemics of trichinellosis, sporadic individual or familiar cases were reported. The main reason of repeated human epidemics in Slovakia has been the permanent circulation of *Trichinella* spp. in sylvatic cycle, especially in red foxes and wild boars. High population density of both animal species, persistent prevalence of trichinellosis in wild boars and even increasing positivity of red foxes suggest that the risk of human outbreaks in Slovakia persists.
O FBP 5-6
The results of investigations on protein profile of *Trichinella* isolates using MALDI-TOF MS
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Trichinellosis caused by *Trichinella* parasite still constitutes a problem, both in Poland and other countries of the European Union. The disease is caused nematodes belonging to the family *Trichinellidae*. So far, nine species and three genotypes of *Trichinella* were classified. To date, the differentiation of the larvae to the species and genotype level is based primarily on molecular methods. In recent years, matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has evolved to a routine method for bacterial species identification due to its comparability to molecular methods, rapidness etc. Therefore, for fast identification of *Trichinella* species and investigation of infection chains, MALDI-TOF MS has recently been developed at the BfR. This is an ionization technique allowing mass spectrometry analysis of biomolecules such as proteins. As both the protein composition of each organism and the MALDI–TOF MS spectrum are unique, the obtained spectra can be compared with a database of known microorganisms and the sample identified with high confidence. The purpose of the study was to analyse obtained spectra from field isolates from wild boars and red foxes gained in Poland in comparison to spectra of isolates from Germany.

*Trichinella* larvae were isolated from 29 wild boars and 2 red foxes from Poland. The species of larvae was determined by multiplex-PCR according to a method of the European Reference Laboratory for Parasites. Proteins from the collected field isolates of wild boars and red foxes from Poland were extracted at the NRL for *Trichinella* (BfR) according to the home made protocol. The obtained using MALDI TOF MS spectra from Polish field isolates were compared with the master spectra library (MSP) generated at the BfR using Flex Analysis software. Master spectrum dendrogram cluster analysis was carried out with the correlation distance measure and single linkage. Score values function was used as a measure of the reliability of the isolate identification.

All obtained spectra from examined Polish field isolates were identified on species level with high likelihood as demonstrated by score values ranging from 2.0 up to 2.5. The larvae from 20 isolates were identified as *T. spiralis* and from 11 isolates as *T. britovi*. Findings confirm that species identification of larvae was possible by means of MALDI TOF MS and in accordance with results previously gained by multiplex-PCR. The investigations proved the usefulness of MALDI TOF MS for fast species identification of *Trichinella* field strains.

Detailed comparison of the spectra of the field *Trichinella* strains from West Pomeranian region of Poland with spectra of *Trichinella* isolates from Mecklenburg–Western Pomerania, Germany showed some differences in protein profiles on the species level. The calculation in FlexAnalysis 3.4 software showed score values that ranged from 1.5 to 1.7, which indicate quite high differences between isolates from Poland and Germany. This finding could indicate the existence of different strain characteristics of *T. spiralis* or *T. britovi* in Poland and Germany. Further detailed investigations should be conducted for proving this hypothesis.
O FBP 6-1

Diagnosis and management of echinococcosis – Romanian experience
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Background: Cystic and Alveolar echinococcosis (CE and AE) are neglected diseases, with a great potential of spreading in Europe, in spite of the existing possibilities of surveillance and control and considering that human to human transmission is not possible. However, the number of the cases by country is increasing, considering the hospital’s data, but the official notification in many European countries is lower.

Aim: presentation of Romanian experience in case identification, diagnosis, management and control of CE during the last 3 years.

Results: Case identification can be done either actively, by US screening in rural population, from remote areas (127 positive cases out of 7469 screened people) or by enrolling the new cases admitted to the hospital (309 patients, with 482 cysts). For all the cases, identification of the major risk factors is analysed.

Imaging methods should be completed with serology (screening and confirmatory tests), carefully taking into account the limits of serology for echinococcosis (young cysts, young patients, calcified cysts, rare locations of the cysts etc.). It is necessary to improve the accuracy of the diagnosis tests.

Case management is taking into account the cysts’ characteristics and patients’ condition or desire: albendazole alone, albendazole associated to surgery or minimally invasive procedures or watch and wait. Analysing the material obtained after surgery or percutaneous techniques by molecular methods, an actual map with the distribution of different genotypes in Europe could be set up. On the other hand the morphology of the cyst can be analysed and correlated with the different genotypes.

In conclusion, the necessity of other pharmaceutical combinations (albendazole plus praziquantel, or cyclodextrine) or new molecules, more active, is obvious. The notification of the cases and the inclusion of the cases in European data base will emphasize the high number of cases. Serum and cyst samples (hydatid fluid, membranes) should be stored for further investigations and projects, in special bio-banks.

The review of the guidelines for the diagnosis and management of echinococcosis seems to be necessary. Sustained control measures should be applied, and veterinarians and human doctors should have a strong collaboration. The same model can be adapted to AE.
O FBP 6-2
Specific serology for *Echinococcus multilocularis* in Dutch patients - COST FBP 2016
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Since 2008 the first autochthonous case of *Echinococcus multilocularis* was detected in the Netherlands (van Dommelen et al, JCM, 2012) three other patients have been detected. All cases had big lesions and were confirmed by PCR of cyst material. However, serology could not detect specific antibodies in the majority of cases. We tested 2 commercially available kits to detect antibodies against *E. multilocularis* from serum. Below are their characteristics.

Bordier Affinity kit: ELISA the EM2+ kit; first positive titer 1:200; costs per kit: (96 wells): ca. €500 (12x8); price per sample (testing in duplo, two strips per sample including controls): €45-90; testing per batch 40 samples: per sample: €12.50.

LDBIO Western blot (WB) kit: difficult to read; in negative an RC bands very close to specific 26-28 band; 16-17-18 difficult to distinguish; kit with 12, 24 or 96 strips ; ca. €600/24; price per sample (always including negative and positive control): €50-75; testing per batch 24 samples per sample: €28.

Table 1: Presentation of serology results tested with Bordier Affinity and LDBIO kit.

<table>
<thead>
<tr>
<th>Patient</th>
<th>F/M</th>
<th>Age</th>
<th>Titer 1</th>
<th>Interpretation</th>
<th>7</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>26-28</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 08-16</td>
<td>F</td>
<td>55</td>
<td>&lt;200</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>2008 09-08</td>
<td>F</td>
<td>56</td>
<td>&lt;200</td>
<td>neg</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>E. granulosus</td>
</tr>
<tr>
<td>(second sample)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011 11-12</td>
<td>F</td>
<td>69</td>
<td>3200</td>
<td>pos</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>E. multilocularis</td>
</tr>
<tr>
<td>2011 11-15</td>
<td>F</td>
<td>69</td>
<td>&lt;200</td>
<td>neg</td>
<td>na</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016 16-13</td>
<td>F</td>
<td>21</td>
<td>&lt;200</td>
<td>neg</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>undetermined</td>
</tr>
</tbody>
</table>

We also performed *Echinococcus granulosus* serology on all patients. In house developed ELISA using sheep cyst Ag and in house developed WB were used.

Table 2: *E. granulosus* serology tested with 2 in house developed kits.

<table>
<thead>
<tr>
<th>Patient</th>
<th>F/M</th>
<th>Age</th>
<th>Titer 1</th>
<th>Concl.</th>
<th>WB IgG1</th>
<th>WB IgG4</th>
<th>Concl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 08-206</td>
<td>F</td>
<td>55</td>
<td>&lt;40</td>
<td>neg</td>
<td>pos</td>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>2008 09-63</td>
<td>F</td>
<td>56</td>
<td>&lt;40</td>
<td>neg</td>
<td>pos</td>
<td>neg</td>
<td>pos</td>
</tr>
<tr>
<td>2011 11-223</td>
<td>F</td>
<td>69</td>
<td>1280</td>
<td>pos</td>
<td>pos</td>
<td>wk pos</td>
<td>pos</td>
</tr>
<tr>
<td>2011 11-249</td>
<td>F</td>
<td>69</td>
<td>320</td>
<td>pos</td>
<td>pos</td>
<td>pos</td>
<td>pos</td>
</tr>
<tr>
<td>2016 16-75</td>
<td>F</td>
<td>21</td>
<td>&lt;40</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
</tbody>
</table>
For Dutch patients the specific *E. multilocularis* ELISA and WB show a very low sensitivity and cannot be used for detecting or follow up patients with alveolar echinococcosis.
Four parasite species of tapeworms that belong to the genus *Echinococcus* are known as causative agents of human disease. *Echinococcus granulosus*, a cause of cystic echinococcosis, shows a cosmopolitan distribution with endemic foci on all continents except Antarctica. It is estimated that approximately 95 percent of human echinococcosis cases worldwide (from a total estimate of three million) are caused by *E. granulosus*. Prevalence and distribution of this organism largely depends on the presence of its intermediate hosts – sheep, cattle, horses, pigs, goats, yaks and camels, as well as the close contact with dogs which serve as the main definite hosts that ultimately transmit the infection to humans. In endemic regions incidence rates for cystic echinococcosis in humans can exceed 50 per 100 000 person-years, with prevalence levels as high as 5-10%. In livestock, the prevalence of cystic echinococcosis found in slaughterhouses in hyper-endemic areas can vary from 20 to 95%. There are at least ten distinct genetic variants (G1 to G10) of *E. granulosus*, with seven of them confirmed to be infectious for humans. The strain most often associated with human cases seems to be the sheep strain or G1.

Morphologically, the adult form of the parasite *E. granulosus* is small in scale (3-7 millimetres in length) and belongs to the family *Taeniidae*. Its body is divisible into scolex, a short neck and strobila that consists of three proglotids: one in immature, one in mature and one in gravid stage. An infected dog may harbour hundreds of adult tapeworms in its intestine where they can live for up to two years. Infection in humans can ensue after accidental ingestion of the tapeworm egg. Once they are out in the environment, these eggs are highly resistant and can remain infectious for several months, and in some cases more than a year if the conditions are moist. While the temperature between 60 and 80°C is known to kill the eggs, they can easily survive freezing temperatures.

When the eggs are swallowed by the intermediate host, the liberated onchosphere penetrates the intestinal mucosa to reach the general circulation and, through blood, different parts of the body where cysts are formed. The chief organs where such cysts are found are liver and lungs, while in rare instances spleen, kidney, heart, bones, eye and central nervous system can also be affected. These cysts may be present in large numbers and they sometimes differ in size and shape. Incubation of the disease may stretch over several years, while clinical manifestation and prognosis depend on size and location of the cysts. Accordingly, cysts that are present in the brain or the eye may lead to severe symptoms and fatal outcome (even if they are small). Although cystic echinococcosis is rarely a lethal disease deaths due to anaphylactic shock or secondary echinococcosis have been described.

Hydatid cysts should be excluded when evaluating patients with cystic disease of any organ. Eosinophilia – often considered a hallmark of certain parasitic infections – is observed in only one fifth of all cases. Pervasive use of radiologic diagnostic is responsible for increased detection of cysts. Laboratory confirmation is typically done by serological tests that have acceptable sensitivity and specificity.
The treatment of cystic echinococcosis can be complicated and expensive, sometimes requiring repeated surgeries and long-term drug therapy. Cystic echinococcosis in humans has been a mandatory reportable illness in Croatia, where up to twenty new cases per year are noted (most notably in the coastal part of the country where sheep raising is widespread due to dietary habits). Laboratory tests are often undertaken for patients in whom liver cysts were discovered by happenstance, most commonly during general check-ups, but sometimes also as a part of diagnostic workup of symptomatic patients. A relatively favourable epidemiological situation in Croatia could be further improved by more rigorous public health preventive measures and via better cooperation between veterinary and human medicine, as well as by educating the public.
Only a few foodborne trematodiases pose serious public health problems in Europe. The most important are fascioliasis caused by *Fasciola hepatica* and opisthorchiasis caused by *Opisthorchis felineus*. Autochthonous rare human infections have been sporadically reported by other trematode species, including infections by *Dicrocoelium*, *Philophthalmus*, *Alaria* and *Heterophyes*. However, reports on human infections by other foodborne trematodes acquired outside, in non-European endemic countries, whether by European travellers or by non-European immigrants or visitors, appear to be more or less numerous depending on the European countries and including infections by highly pathogenic species such as *F. gigantica*, *O. viverrini*, *Clonorchis sinensis*, and *Paragonimus* spp. besides other secondary species rarely found in humans. In Europe, the geographical distribution of *F. hepatica* covers almost the whole continent, with the exception of the northern latitudes of the Scandinavian countries, giving rise to a wide veterinary problem in livestock. However, *O. felineus* infects several species of fish-eating mammals in southern, central, and eastern Europe, e.g. Italy, Albania, Greece, Switzerland, The Netherlands, Germany, Poland, the European part of Russia, Ukraine and Bielorussia, with human reports from the latter three countries and recently also from Italy. Diagnosis in humans and animals usually relies on egg identification in stools, although adult stage morphology may be used in species or ectopic infections affecting organs not related to the digestive system, such as in ocular cases by *Philophthalmus* and *Alaria*. A few highly specific immunological tests have been developed for the most important species, including *Fasciola*, the opisthorchids and *Paragonimus*, although care with cross-reactions should always be considered. Concerning food analyses, detection methods of foodborne trematodides include from traditional microscopic methods up to recent different molecular techniques. Although the identification of worms remains as the gold standard method for the detection of trematodes in food, molecular and immunological tools appear to be progressively increasing. The detection and identification of plant-attached and tissue-encysted metacercariae mainly rely on the morphological characterization by visual inspection and the use of methods ranging from direct vegetable or tissue examination to mechanical or enzymatic tissue disruption. In the case of trematodes using fishes as second intermediate host, the examination of metacercariae is usually performed by muscle compression or by pepsin-HCl artificial digestion. For the identification of the metacercariae, whether at generic or specific level, they should be observed in detail under a light microscope and whenever possible classified based on characteristic features and dimensions. Important characteristic features include the shape of the cysts, presence and size of suckers and spines, and shape and contents of the excretory bladder. In cases in which the morphological features cannot be distinguished or are difficult to be observed, metacercariae should then be excysted. Metacercariae can be easily released with slight pressure when the cyst wall is very thin. An artificial digestion may be performed when the cyst is thick. There are several methods available for the digestion of the metacercarial cysts. They mainly involve metacercarial incubation in trypsin or bile at 37°C. The application of molecular methods for the detection of metacercariae in foods is still very limited, but increasing progress indicates that they may become useful tools in the near future.
Contribution of molecular identification of *Diphyllobothrium*

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Tapeworms of the genus *Diphyllobothrium* (Cobold, 1858) are widely distributed all around the world and some of them are agents of human diphyllobothriosis. Approximately 50 species have been described within the *Diphyllobothrium* genus but only 14 are human pathogens (Scholz et al., Clin Microbiol Rev 2009; 22: 146-160). The parasite is transmitted after consumption of raw or undercooked fish. When symptomatic, diphyllobothriosis usually manifests as intestinal disorders (abdominal discomfort, abdominal pain, diarrhoea, transit disorders, and constipation), weight loss, asthenia, and vertigo. The diagnosis of human infection is based on the identification of proglottids expelled with the stool or of eggs after stool examination. As species identification by using morphological criteria is difficult, we determined that cytochrome c oxidase subunit 1 gene (*cox1*) sequences analysis was a useful tool for identifying specimens (Yera et al., Parasite 2008; 15: 402-407). Using molecular identification, it was possible to diagnose human exotic diphyllobothriosis cases acquired after travel or consumption of imported fish, and to avoid misidentification of *Diphyllobothrium* with other Cestoda (Yera et al., Parasitol Int 2006; 55: 45-49; Paugam et al., La Presse Médicale 2008; 38: 675-677; Yera et al., Parasitol Int 2013; 62: 268-271). Molecular identification of isolates from human or contaminated fish is recommended to correctly determine the epidemiology of diphyllobothriosis.
Experimental model of chemoprevention in hepato-peritoneal cysticercosis in pigs
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The reservoir for hepato-peritoneal cysticercosis is represented by dogs, particularly shepherd and hunting dogs, whereas the intermediate host is represented by sheep, cattle, goats, pigs, rarely horses. In natural infestations, a series of uncharacteristic manifestations have been observed. When the cysticerci number is high, apparent disorders of hepatitis are developed, which may be complicated by acute peritonitis and ascites. In the liver and peritoneum, bleeding with fatal outcome may occur. In experimental infestations, an anaemia syndrome and fever with the onset on the third day from contamination have been observed. Light infestations cause only chronic anaemia with weakening and eosinophilia.

The treatment and chemoprevention in hepato-peritoneal cysticercosis in animals have received only little attention. Therefore, the aim of the present study was to develop an experimental model of chemoprevention in hepato-peritoneal cysticercosis in pigs, by testing the chemopreventive value of a benzimidazole derivative: albendazole.

The experiment was conducted on 10 Romanian Saddleback (Bazna) pigs (age 3 months, body weight 25-35 kg at the beginning of the experiment) within April–June, 2015 (60 days). Each pig was administered orally 4000 oncospheres of *Taenia hydatigena* from a dog, for two consecutive days. The activity of albendazole (2 mg/kg body weight/day/animal) was tested in 5 pigs (experimental group) compared to an untreated infested group (5 pigs, control group). The two groups were weighed and examined clinically during the entire experimental period.

Blood samples were collected on days 0, 16, 30, and 45 of the experiment, and analysed for the following parameters: erythrocyte count, hemoglobin, leukocyte count, total proteins, serum albumin, gamma globulins, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (AP). On day 50 of the experiment and three days after the end of the experiment, respectively, one pig from each group underwent laparoscopy and ultrasound, for diagnostic purposes.

The research on the chemoprevention in hepato-peritoneal cysticercosis in pigs showed the following: (*) from the statistical point of view, there were no differences between the two groups on the values of analysed blood parameters; (*) the administration of albendazole showed only partial protection in serous cysticercosis, in the experimental group compared to the control group; (*) laparoscopy has a diagnostic value in experimental hepato-peritoneal cysticercosis in pigs, but is a traumatic exploratory method; (*) ultrasound scan is an effective, modern, non-traumatic and rapid method for the diagnosis of serous cysticercosis in swine.
Assessment of the computer-based *Taenia solium* educational program ‘The Vicious Worm’ on knowledge uptake in primary school children in Katete district in Eastern Zambia

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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 assess the impact of the computer-based *T. solium* educational program ‘The Vicious Worm’ on knowledge uptake in primary school aged children in a highly endemic region of Zambia.

Children from grades five and six (age range 10-18 years) at Nyembe Primary School in the Katete district of eastern Zambia were invited to attend a Saturday morning workshop at the primary school in July 2016. A laptop and projector were used to administer a twenty-four question multiple-choice quiz, in both English and Chewa, to the children, who used TurningPoint© clicker devices to submit their answers in real time. Following this, the introductory and Village parts of The Vicious Worm program were presented to the students in Chewa for approximately one hour. Finally, the children were exposed to a rearranged version of the original quiz to assess their knowledge uptake.

In total, 40 children participated in the workshop: 11 males (27.5%) and 29 females (72.5%), with a median age of 13 years (range 10-18 years). Baseline knowledge of human taeniasis (TS) was 74.3%, of diagnosis of porcine cysticercosis (PCC) was 68.8%, of the relationship between PCC/TS and neurocysticercosis (NCC) was 55.3%, of *T. solium* acquisition and transmission was 55.3%, of NCC in general was 44.3%, of PCC/TS/NCC prevention was 42.7%, of PCC treatment was 35.0%, and of acquisition of NCC was 26.5%. Immediately after exposure to ‘The Vicious Worm’, knowledge increased in all of the categories except for the acquisition of NCC, which slightly decreased. Knowledge relating to acquisition/transmission of *T. solium* infections and of NCC in general both increased by 25% or more.

The initial questionnaire results indicated that the baseline knowledge of surveyed school children regarding *T. solium* TS/CC was generally rather good, which is likely due to the success of the ongoing CYSTISTOP project activities in this community.

Preliminary assessment of ‘The Vicious Worm’ indicates that it is an effective *T. solium* educational tool for primary school aged children in Zambia. Follow-up studies are planned to assess the longer-term
impact of the program on knowledge uptake in this area. Inclusion of ‘The Vicious Worm’ workshops in endemic areas should be considered for inclusion in integrated cysticercosis control and/or eradication campaigns.
Introduction: Over 50 million people suffer from epilepsy worldwide with more than 80% in developing countries. Neurocysticercosis (NCC) is the most common helminthic infection of the central nervous system, causing more than one quarter of acquired epilepsy in endemic areas. Northern Uganda is highly endemic for *Taenia solium*, the perpetrator of NCC. This study evaluates the prevalence and risk factors for NCC in northern Uganda.

Methods: Between 2009 and 2012, we identified individuals with neurologic signs of epilepsy in northern Uganda using door to door systematic random sampling. A subset of identified epilepsy cases, selected based on seizure recency, was further interviewed with the CWGESA questionnaire and screened for NCC based on the Del Brutto criteria. Risk factors for NCC were studied using univariable and multivariable logistic regression models.

Results: Out of 38,303 screened individuals, 1,245 epilepsy patients were identified (3.3% [CI: 3.1–3.4]). Out of the 300 people with epilepsy who underwent further screening, 40 were diagnosed with NCC (13.3% [CI: 10-18]). The infection risk increased by 4% per year of life lived (aOR=1.04 [95%CI: 1.01–1.07]; p=0.013). Farmers had a higher risk of NCC than students (OR=0.18 [95%CI: 0.07–0.43]; p<0.001), however pork consumption was not found to be a risk factor (p=0.337). There was a suggestion that education may play a protective role (aOR=3.22 [95%CI: 1.33–8.23]; p=0.011). Especially seizure onset, seizure type, headache type and possibly pre-existing conditions were identified as statistically relevant. Clinical risk factors included: tape worm carrier status (OR=62 [CI: 16-411] p<0.001), past medical history (pmh) of cerebral malaria (OR= 0.42 [95%CI: 0.17-0.95] p=0.035) and pmh of meningitis (OR=2.49 [95%CI: 1.16-5.14]; p=0.02). Headache history was borderline statistically relevant (OR=2.44 [95%CI: 1.01–5.52]; p= 0.049).

Epilepsy specific information contained some statistically relevant information that may help characterize NCC specific epilepsy in the future.

Discussion: A list of factors will help identify a “typical NCC patient” through further defining headache, seizure patterns and other sociodemographic factors. Clinical prediction rule might be an option for pre-screening in resource poor countries. As risk factors vary from one country and culture to another, it is important to understand the specific risks involved with attaining NCC in Uganda. This may help establish a sounder public health intervention in the future. As data quality and funding was limited, new studies should try and extend the number.
Fact sheets for strategic communication to the general public on *Taenia solium* and *Taenia saginata*

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An important part of the current focus areas in research are aimed at preventing diseases, that includes understanding the development of *Taenia* species, the host parasite interactions, transmission, distribution, and ways to control the effects they pose on animals, humans, and the environment. One aspect that is often forgotten in research activities is bridging the gap between scientific research and the general public. More efforts should be devoted to create increased awareness among the general public to widen the impact on society and understand why research is needed. Although the Internet is widely accessible correct, easily understandable information is still sparse and not widely available. Our aim was to develop fact sheets for *T. solium* and *T. saginata* for strategic communication and information dissemination to the general public. The fact sheets contain the most recent knowledge and reviews of the studies published on the parasites. They are written in a clear understandable language to laypersons. The fact sheets are targeted toward those who would like to understand and learn more about the diseases caused by the pork and the beef tapeworms. The fact sheets will be published on the CYSINET and the WHO homepages. The fact sheets are optimised to appear by simple Google search in order to reach a wider audience. They will be available in a printable format to be used for informative purposes in other contexts, e.g. presented as posters in schools or during information campaigns on food safety.

The work presented was done in collaboration with CYSTINET members, COST Action TD1302
Disease behaviours of sows naturally infected with *Taenia solium* in Tanzania

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Neurocysticercosis (NCC), the parasitic infection of the central nervous system caused by the zoonotic parasite *Taenia solium*, is a serious neurological disease in pigs and humans. The impact of the disease on pig behaviour has so far been little explored. The aim of this study was to assess and describe the effect of NCC on active, passive and feeding behaviours as indicators of reduced welfare in naturally infected sows.

In total 13 *T. solium* naturally infected and 15 non-infected control sows were videotaped for two consecutive weeks using close circuit television cameras. Videos were analysed at three different time points, namely at the beginning, in the middle and at the end of the two week recording period. For each time point, videos were analysed at 3 defined time periods, namely feeding, enrichment period and during a whole day.

The results of the study indicated that NCC changed the behaviour of diseased sows. Sows with NCC spent significantly less time at the feeding trough, especially during the second half of the feeding period, were more passive e.g. lying and standing still significantly more during a whole day period and showed social isolation compared to non-infected control sows. From an animal welfare perspective these results emphasize the need to control the disease in areas where the disease is endemic through both, prevention by educating farmers and where possible treatment of diseased animals. Interdisciplinary co-operation through a One Health approach will be needed in order not to further neglect the animal welfare aspect of this zoonotic disease.
Diagnostics development pipeline

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O FBP&CN 2
What types of information can be gleaned from both whole genome and targeted amplicon NGS data of pathogens?
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The technological revolution in genome and other sequence generation methods has been astounding. There is much promise for what the technology holds but also many real world concerns about the applicability or even the necessity of NGS methods when other detection and identification methods exist. This talk will examine a few parasite systems in which NGS approaches were employed and discuss what was learned. In reality, one size will not fit all and the stage should be set for a healthy discussion of both the challenges and the opportunities facing parasite and food-borne pathogen communities.
O FBP&CN 3
Ranking foodborne parasites in Europe using multicriteria decision analyses.
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In 2012, 24 foodborne parasites (FBP) of global importance were identified and ranked by FAO using multicriteria decision analyses (MCDA). Taenia solium ranked highest, but as this might differ regionally, it was recommended that ranking on a regional level should also be carried out (FAO/WHO, 2014). One aim of Work Package 1 within COST Action FA1408 (EURO-FBP) was to rank the FBP relevant for Europe using the same methodology as was used in 2012. Moreover, we also aimed to rank the FBP for the different regions of Europe to obtain information about the regional importance of FBP. The results of this ranking exercise will be used to identify gaps in available data and to advice on surveillance systems and a research agenda on FBP in Europe.

From 8-12 February 2016, 33 experts of the EURO-FBP COST Action attended a five-day workshop at RIVM in Bilthoven to rank FBP in Europe using MCDA. Prior to the workshop, they had filled in a questionnaire with the aim of identifying additional FBP and criteria. In addition to the same nine criteria and 24 FBP as used during the global ranking (FAO/WHO, 2014), one additional criterion (the probability of introduction) and one additional parasite (Angiostrongylus cantonensis) were discussed and included in the EURO-FBP workshop. Weights indicating the relative importance of each of the 10 criteria were assessed during the workshop. All 25 FBP were scored in subgroups for the 10 criteria, using precise decision rules. The scores were summed and normalised between 0 and 1 to compare the ranking of the 25 FBP at the Pan-European level and for the five different European regions as scored in subgroups. At the Pan-European level, Echinococcus multilocularis ranked first, followed by Toxoplasma gondii, Trichinella spiralis, Echinococcus granulosus and Cryptosporidium spp. At the regional level, E. multilocularis also ranked highest in Northern and Eastern Europe, but in Western Europe Toxoplasma gondii ranked highest. Whereas in both Southwest and Southeast Europe, E. granulosus ranked highest.

In summary, other FBP are of importance to Europe compared to the world. When comparing the results of the Pan-European ranking with the global ranking, E. multilocularis was ranked first and T. gondii second. Anisakidae were ranked 17th on a global level (FAO/WHO, 2014), but appear in every regional top ten, as well as the Pan-European top ten. Only Cryptosporidium spp. held the same place in the top ten of most important FBP. At the European regional level, differences in ranking indicate that the regional variations should be considered when surveillance systems are defined. For instance, E. granulosus may not seem as a very high priority at the Pan-European level, but should be considered as very important in Southwestern and Southeastern Europe. The next task for WP 1 will be to study
gaps in Pan-European and regional surveillance systems, taking into account the results of the Pan-European and regional rankings.
O FBP&CN 4

Contribution of PCR in Cerebrospinal Fluid Samples for the diagnosis of Neurocysticercosis in Patients living in a non-endemic country
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Neurocysticercosis diagnosis is based on a combination of clinical, epidemiological, radiological, and immunological findings (Del Brutto et al., Neurology 2001; 57: 177-183; Del Brutto, Pathog Glob Health 2012; 106: 299-304). The disease is endemic in developing countries. In France, infection via wild boar meat is a possibility, but autochthonous cysticercosis is very rare (Duong et al., Presse Med 2006; 35: 243-245). We used a real-time PCR assay for the confirmation of neurocysticercosis diagnosis in cerebrospinal fluid from patients living in France (Yera et al., J Clin Microbiol 2011; 49: 4338-4340). The assay allowed the confirmation of neurocysticercosis in patients that had traveled to tropical countries more than 7 years before symptom onset. The assay had 100% specificity and sensitivities of 83% in patients suspected of neurocysticercosis or 93% in patients with a definitive diagnosis of neurocysticercosis. It avoided the need for brain biopsy. Moreover, it might be useful for disease and treatment follow-up.
Microfluidic devices for parasitology
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Each year more than 600 million foodborne sickness is reported worldwide, with 40% of hit in children younger than 5 years. In 2010, according to World Health Organization (WHO), more than 23 million get illness from foodborne parasites. Due to the effects of globalization, today the breaking out of epidemics is not only in the backward areas, but in the advanced countries too. In Europe, the number of the observed cases is increasing.

Based on the emerging importance of foodborne parasitology, novel diagnostic methods and tools have been developed. One of theirs realization, which is called “Lab on a chip” (LOC), miniaturizes a complex laboratory into an integrated LOC device. These instruments operate with biological samples in the range from few picoliter up to millilitre. In laboratory protocols generally, the detection of the foodborne parasites has been overtaken by sample preparation and the enrichment of pathogens. The microfluidic devises could integrate also sample pre-treatment steps to concentrate pathogens from biopsy, liquor, stool, or samples for serological examination.

The observation of parasites is based on microscopy and molecular biological methods. One of another advantage of microfluidic devices could be to integrate these techniques together. Using the microfluidic devices the pathogens could be filtered out from the sample, analysed optically, than open the pathogens, extracts their genomes and do any kind of PCR within the same device. In those detection cases, when the population of the pathogens per volume is low in the samples, microfluidic devices could be applied efficiently to enrich pathogens from a bigger volume (the range from millilitres up to litre) and to detect parasites directly inside the microfluidic structures.

Using microfluidic devices, we could successfully detect parasites from blood and biopsy samples. We would like to demonstrate our recent results on the detection of foodborne parasites (e.g. Trichinella spices) and other bloodborne parasites (e.g. Dirofilaria spices) in our oral presentation. The developed microfluidic devices will be also presented in the poster section.
O FBP&CN 6
Evaluation of 2B2t recombinant antigen in western blot for diagnosis of cystic echinococcosis
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Background: Human cystic echinococcosis (CE) diagnosis and monitoring are carried out primarily by an imaging technique (most frequently ultrasonography) and with an immunodiagnostic test as adjunctive. Current serological techniques are based on the detection of antibodies against crude hydatid fluid (HF) or partially purified antigens from HF. These crude antigens show some drawbacks, including low sensitivity for some CE patients and low specificity and cross reactions with other parasitic diseases. Numerous recombinant antigens have been proposed as substitutes of the HF for the diagnosis of CE in order to solve these drawbacks. A recombinant antigen derived from the antigen B2 (2B2t) led to promising results in an ELISA test for the diagnosis and monitoring of CE patients. Here, we evaluate the performance of this recombinant protein 2B2t in western blot (WB) and compare these results with an ELISA using HF.

Materials and methods: The recombinant antigen 2B2t was expressed and purified as it is described in Hernández-González et al., 2012. After establishing the optimal conditions for the 2B2t in WB using an anti-human total IgG conjugate, we tested the following serum collection: 142 samples from CE patients; 126 sera from CE negative individuals, of whom 49 were donors (38 were Spanish and 11 were Latin American), 36 sub-Saharan African patients with eosinophilia and negative serology to several parasitoses and 4 subjects with no parasitic diseases that were positive to a commercial HAI test for CE. In addition we also tested 29 sera from individuals with neurocysticercosis (NCC) and 8 with schistosomosis. The sensitivity and specificity were calculated by calculating the percentage of true and false positive results.

Moreover, the sera from CE patients were tested through an “in-house” ELISA assay for IgG1 and IgG4 detection against HF as described previously (Güerri et al., 2000), considering positive those samples with ODs above the cut off calculated for either of the two isotypes.

Results: 2B2t in WB showed a sensitivity of 74.6% (106 positive samples). In comparison, the ELISA using HF detected just 55.6% of the samples being positive to IgG1 (20 sera) and IgG4 (69 sera). The specificity of 2B2t in WB was 96.8%. False positives were found for 2 Venezuelan patients with NCC who also presented taeniosis and one patient with schistosomosis. Also, a serum from a sub-Saharan African patient was positive in this assay although it is difficult to say whether he has actually been in contact with the parasite or not. To date, the ELISA-HF specificity could not be tested with the same collection of sera.

Conclusion: The application of the recombinant 2B2t in a WB assay has a good specificity and sensitivity for CE sero-diagnosis and show advantages, higher homogeneity and excellent reproducibility among others, when compared with the use of a crude antigen (HF). This protein, combined with other Echinococcus granulosus recombinant antigens that complement its diagnosis properties, could be a relevant alternative for the disease immunodetection.
Development of LAMP-based procedure to detect *Toxoplasma gondii* oocyst in ready-to-eat salad

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An ISO standard for the detection of *Toxoplasma gondii* oocysts in water, fresh fruits and vegetable is not available. Moreover, the direct identification of *T. gondii* oocysts by microscopy is not reliable due to the low number of oocysts often present in the sample and to high similarity with oocysts from other coccidian species and the lack of specific antibodies. Molecular detection of *T. gondii* DNA is a valuable strategy but it is still challenging, mainly due to the lack of sensitivity. Sensitive technique such as qPCR, are available but require expensive instruments and reagents limiting its use to well-equipped laboratories. As a consequence, toxoplasmosis is considered to be an under-detected and under-reported disease. The development of a sensitive, accurate and easy to perform molecular method is then essential to ensure the correct identification of this pathogen. The loop-mediated isothermal amplification (LAMP) has been proved to be as sensitive as qPCR and has been successfully used to detect the parasite oocysts in water and soil. Here we setup a molecular protocol to detect *T. gondii* in ready-to-eat salad (baby lettuce). The protocol combines the procedure defined in the ISO 18744:2016, for the detection of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables, with FastPrep system for DNA extraction and the amplification of a highly repeated and specific 529 base pair fragment, by loop-mediated isothermal amplification (LAMP). LAMP products are finally analysed by agarose gel electrophoresis. The reaction is specific and no amplification was observed when human, vegetable and other parasite DNAs were used. The sensitivity of the method allows the detection of 10 fg of pure *T. gondii* gDNA. The LAMP allow the detection of less than 10 oocysts in 1 ml of pellet obtained from 50g of washed vegetable and down to 25 oocysts spiked in 50g of vegetable. When *T. gondii* positive vegetable samples were tested by the real-time PCR targeting the same 529bp sequence, the sensitivity was comparable to LAMP. Finally, a Lateral Flow Dipstick (LFD) chromatographic detection method is under evaluation for a rapid visualization of the LAMP result without gel electrophoresis.
The detection of *Toxoplasma gondii* in milk of goats from Poland by PCR methods

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The potential transmission of *Toxoplasma gondii* by unpasteurized goat milk has been suggested as a risk factor for toxoplasmosis in humans. The aim of the study was preliminary comparison the efficiency of selected PCR methods to detect *T. gondii* DNA in goat milk.

In total, milk samples of 60 lactating females from 35 farms localized in West Poland were collected. The samples were centrifuged, and to avoid interference by casein, the pellet of milk was treated with TE and EDTA buffers, according to the method described by Psifidi et al. (2010). DNA from samples of milk was extracted using QIAamp DNA mini kit (Qiagen) according to the manufacturer’s instruction. DNA isolation was performed also for part of milk pellets without buffers treatment. For detection of *T. gondii* DNA (B1 gene) in milk samples, nested PCR (by Grigg and Boothroyd, 2001) and Real time PCR (RT PCR, by Lin et al., 2000, treated with TE and EDTA buffers samples only) was performed. In next step, selected positive samples in nested PCR (23 samples) were genotyped by multilocus PCR using 6 genetic markers: SAG1, 5′-SAG2, 3′-SAG2, SAG3, BTUB and GRA6 (Dubey et al., 2006). Amplicons were sequenced and compared with the NCBI database using Blast.

In total, among examined 60 milk samples (treated with buffers), 26 (43.3%) and 39 (65%) of them were positive in nested PCR and RT PCR, respectively (kappa value=0.762). Examination of untreated milk samples in nested PCR, showed only 8 (13.3%) positive results. The analysis of multilocus PCR for nested PCR positive samples, revealed the occurrence of genotype type III for all samples. However, depending on the sample, genotyping was possible based on 1-4 markers, only.

In conclusion, detection of *T. gondii* DNA in many milk samples (43.3-65%) can confirm the role of goat milk as the potential source of *T. gondii* infection for humans in Poland. Preliminary assessment of few PCR methods for detection of *T. gondii* DNA in goat milk can confirm the need of dissolve milk casein before extraction of DNA to the rise of PCR efficacy. Despite of the higher sensitivity of the Real time PCR compared with nested PCR stated in our study, for some milk samples (probably with more inhibitors) nested PCR can be more suitable (5 samples positive in nested PCR were negative in RT PCR). Because of low concentration of toxoplasma DNA in positive milk samples, the preliminary multiplication of *T. gondii* (e.g. by bioassay in mice) is needed to improve the genotyping.
First confirmed waterborne Cryptosporidium outbreak in Hungary linked primarily to children's pool and jacuzzi

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Cryptosporidiosis is a gastroenteric disease caused by the protozoan parasite Cryptosporidium, which manifests primarily as watery diarrhoea. Transmitted via the faecal-oral route and infection with the parasite can occur through ingestion of water or food contaminated with its infective oocyst stage. In the months of June and July 2015 there were 35 notified cases of cryptosporidiosis (with enteric symptoms) from Somogy county, Hungary. 19 out of 35 patients were transplanted children. Interviews conducted by local government staff on the notified cases revealed that all cases had visited the same Hotel over June 14-20, 2015. There were rehabilitation camps organized for transplanted children and their families, all together for 193 person (49 transplanted).

Case-patients ranged in age from 0 to 60 years; the highest attack rate (54.3%) was for children aged 1-10 years. 12 laboratory-confirmed cases were reported. 11 out of 12 confirmed stool samples were from transplanted children with average age 8.9 (± 2.44).

A case-control study revealed that case-patients were more likely than controls to have swum in the children’s pool (25/29 vs. 9/75; odds ratio [OR], 7.18; 95% confidence interval 2.9-17.2). Case-patients were more likely than controls to have swum in the jacuzzi pool (27/44 vs. 7/60; OR, 5.25; 95% confidence interval 2.1-13.1). Case-patients were also more likely than controls to have put their heads under the water.

A part of the transplanted patients have been treated by Nitazoxanide. Each patient has recovered, which have been confirmed by control laboratory tests.

On site investigation revealed that the pools with a water recirculation system worked properly, irregularities were not found and operations were according to the relevant regulations. Technologies of the children’s pool include aluminium chloride flocculation, sand-gravel filtration and disinfection using sodium hypochlorite (free chlorine 0.3-0.5 mg/L). The complete change of waters have been performed before the camp (14.05.2015). Self-control chemical tests during the camp and complex bacteriological, parasitological and chemical tests performed by the government’s laboratories between 29 June and 2 July were all negative.
P FBP 4

Toxoplasma gondii circulation in game animals in Slovakia
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In Slovakia, there is no official monitoring program for the diagnosis of toxoplasmosis in livestock or free-living animals. *Toxoplasma gondii* surveys are conducted solely to assess an upcoming epidemiological situation or at the request of farmers. Therefore the data on *T. gondii* distribution in free-living animals in Slovakia are very sporadic in game animals are not known at all. *T. gondii* are present in wildlife animal tissues and a consumption of game meat might be a potential source of infection for humans. The aim of the study was to assess *T. gondii* antibody-prevalence in wild boar, red deer, roe deer, and fallow deer intended for human consumption. During 2007-2014, a survey on toxoplasmosis distribution in game was conducted in hunting areas in the Central and Eastern Slovakia. The presence of *T. gondii* antibodies was detected by indirect ELISA in the serum or meat juice of 444 game animals. The highest prevalence was recorded in wild boar (*Sus scrofa*) with 43.9%, followed by red deer (*Cervus elaphus*) with 28.9%, roe deer (*Capreolus capreolus*) with 28.0%, and fallow deer (*Dama dama*) with 5.6%. The high positivity for *T. gondii* antibodies in examined animals indicates a high exposure to protozoan parasite *T. gondii* in the studied localities. A consumption of undercooked or raw meat containing tissue cysts represents a risk of the human infection. The spread of toxoplasmosis in the human population is due to the interchange of the domestic and sylvatic cycles.
Prevalence and diagnosis of parasitic diseases (foodborne parasites) in Bulgaria
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The level of registered parasites in humans in Bulgaria in recent years is with tendency to permanently constant. The results on the prevalence of parasitosis are from the annual reports of the diagnostic laboratories in the country after morphological and immunological examinations. Foodborne parasites (FBP) registered include protozoa, nematodes, cestodes, and trematodes are meat borne, transmitted by water, soil or person-to-person contact. The prevalence of parasitic diseases not changed for the last 3 years. From a total of 782 336 persons tested, 1.63% confirmed a positive result for the presence of various parasites in year 2014. Top line is echinococcosis (average incidence for year - 4.09‰ and mortality - 0.03‰) and trichinellosis (overall morbidity 0.37‰). Soil transmitted parasitoses declined in the last 5 years and the prognosis is that this process will continue. The most common foodborne parasites are protozoa such as Giardia lamblia (0.4%), Blastocystis hominis (0.27%), Toxoplasma gondii (0.36‰) and helminthes as Ascaris lumbricoides (0.10%), Trichocephalus trichiuris (0.02%), Hymenolepis nana (0.03%), Enterobius vermicularis (1.07%). Year registered persons with Taeniarhynchus saginatus are 26 (0.33‰). Bulgaria has a long-standing registration practice of parasitic diseases, but in recent years due to reducing numbers of specialist medical doctors – parasitologists there are difficulties to enumerate number of people with parasitic diseases. To limit the spread of parasitic diseases are necessary comprehensive measures for diagnosis, treatment, prevention and registration.

Bulgaria has a long-standing registration practice of parasitic diseases, but in recent years due to reducing numbers of specialist medical doctors – parasitologists there are difficulties to enumerate number of people with parasitic diseases.
P FBP 6
Production of anti-SAG1 IgY antibody for detection of Toxoplasma gondii parasites
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Specific antibodies are generally useful for direct detection of parasites, both in infected persons and in different kinds of samples, such as infected meat, milk, water, and green vegetable. Parasite specific antibodies are also used in serological methods and scientific researches of host-parasite interactions, parasite invasion and replication mechanisms, characterization of virulence factors. SAG1 is the immunodominant surface antigen in tachyzoite form of T. gondii. SAG1 is the main target of immune response against parasite in hosts. Mammalian antibodies against SAG1 were produced by several researchers and companies. However yolk antibodies have many advantages over mammalian antibodies in many ways like ease of production (non-invasive, rapid, and economical collection system), low costs and antibody production in high quantity. Chicken egg yolk antibody, also known as immunoglobulin Y (IgY), is the predominant class of serum immunoglobulins in birds. In this study, hens were immunized with recombinant SAG1 and T. gondii-specific IgYs were produced. After the purification of produced IgYs by PEG 6000 precipitation method, specificity of produced antibodies was determined by ELISA method and Western Blotting. Protein concentrations were calculated by nanospectrophotometer. Considering the advantages of IgY and importance of SAG1 in toxoplasmosis, anti-SAG1 IgY will play an increasing role and gain commercial value in research, diagnostics, and immunotherapy against toxoplasmosis. Anti-SAG1 IgY antibodies can be labelled with dye markers, enzymes or latex particles for parasite detection with different methods in the future.
The official therapy for taeniosis (*Taenia saginata* or *Taenia solium*) involves administration of praziquantel or niclosamide. The aim of this paper is to review some of the traditional phytotherapy for taeniosis.

Medicinal plants include:
- Pumpkin seeds and pomegranate peel are well known traditional antihelminthics whose use is widespread in Asia, Africa and Europe;
- Garlic is better known as a medicine for nematode infections and may prevent mixed infections;
- Bark of the buckthorn bush and leaves of the senna plant are generally accepted laxatives;
- Valerian root, mint leaves, wild wormwood have anti-inflammatory, relaxant and mild laxative effects.

The efficiency of these mixtures, we do not know.
## List of participants

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