EUROPEAN UNION REFERENCE LABORATORY

FOR PARASITES

WORK PROGRAMME

2012

The 2012 working programme of EURL for Parasites (EURLP) consists of the following activities:

1. Ad hoc activities

1.1 *Trichinella*

1.1.1 To increase and maintain the serum bank of *Trichinella*-infected pigs (multi-years)

1.1.2 To establish a *Trichinella*-positive standard pig serum (multi-years)

1.1.3 To increase and maintain the serum bank of *Trichinella*-infected humans (multi-years)

1.1.4 To produce reference *Trichinella* antigens for serology (multi-years)

1.1.5 Maintenance of reference strains of *Trichinella* in vivo (multi-years)

1.1.6 Screening of commercial kits to detect anti-*Trichinella* IgG in pig sera (multi-years)

1.1.7 Diagnostic activity with accredited methods (multi-years)

1.2 Anisakidae

1.2.1 To increase and maintain the collection of Anisakidae worms and/or their genomic DNA (multi-years)

1.2.2 Diagnostic activity with the accredited method (multi-years)

1.3 *Echinococcus*

1.3.1 To maintain and improve the genetic bank of cestodes of the genus *Echinococcus* (multi-years)

1.3.2 To maintain and improve the serum bank of *Echinococcus*-infected humans (multi-years)

1.3.3 Diagnostic activity with the accredited method (multi-years)

1.4 Other Cestodes

1.4.1 To maintain and improve the genetic bank of zoonotic cestodes such as those of the genus *Taenia* and *Diphyllobotrium* (multi-years)

1.5 Trematodes

1.5.1 To maintain and improve the genetic bank of zoonotic Opisthorchidae trematodes (multi-years)

1.5.2 To maintain and improve the serum bank of *Opisthorchis*-infected humans (multi-years)

1.6 *Cryptosporidium*

1.6.1 To maintain and improve the genetic bank of protozoa of the genus *Cryptosporidium* (multi-years)

1.7 *Giardia*

1.7.1 To maintain and improve the genetic bank of protozoa of the genus *Giardia* (multi-years)

2. Research

2.1 Barcoding of zoonotic and non zoonotic helminths and protozoa parasitizing domestic animals and contaminating foodstuffs (multi-years)

2.2 Identification of *Toxoplasma gondii* proteins specific for the oocyst stage (multi-years)

2.3 Identification of polymorphic microsatellites in *Trichinella spiralis* and *Trichinella britovi* (multi-years)

2.4 Identification of *Trichinella*-specific antigens most frequently recognised by swine sera by Western blot (multi-years)

2.5 Development and validation of a molecular test to identify parasites of the genus *Opisthorchis* (multi-years)

2.6 Development and validation of a serological tests to detect anti-*Opisthorchis* spp. IgG in human sera (multi-years)

2.7 Development of a molecular test to identify at the species level parasites of the genus *Taenia* (multi-years)

2.8 Study of the genetic polymorphisms of *Echinococcus granulosus* sensu stricto (multi-years)

2.9 Development of molecular test to identify *Dientamoeba fragilis* in human and animal faecal samples (two-years)

2.10 Development and validation of an analytical method for the species/assemblage identification of parasites of the genus *Giardia* (one-year)

3 Interlaboratory comparison study

3.1 *Trichinella*

3.1.1 PT on *Trichinella* larva detection in meat samples (multi-years)

3.1.2 PT on *Trichinella* larva identification

3.2 *Echinococcus*

3.2.1 PT on *Echinococcus* adult worms detection

4 Workshop

5 Visit to NRLs

6 Training for Personnel of NRLs and of developing countries

7 Further development and update of the web site of the EURL for parasites

8 Standardization of methods for the detection of parasites in food

9 Development of reference materials for diagnostic methods to detect *Trichinella* infections

10 Validation of apparatuses for the detection of *Trichinella* larvae in meat samples

11 Quality Assurance System

12 Meeting at the DG SANCO

1. Multi-years ad hoc activities

1.1 *Trichinella*

1.1.1 To increase and maintain a serum bank of *Trichinella*-infected pigs

Serum and/or meat juice samples will be collected from *Trichinella*-infected pigs, from pigs infected with other parasites, and from pigs known to be *Trichinella*-free. All samples will be tested by the validated ELISA, distributed in aliquots, lyophilised and stored at +4°C. The database of the serum bank will be updated. Pig serum samples from different world regions and pig races (infected and not infected with *Trichinella*) will be collected, in order to obtain control sera and to refine the most appropriate cut-off value which will be useful for serological studies on different swine races. If the EURLP will receive a high request of reference serum samples, SPF pigs will be experimentally infected with *Trichinella* spp. larvae. Before the infection, sera will be collected from each pig. After the infection, the kinetics of anti-*Trichinella* antibodies will be followed and, when the serum conversion will be detected (approximately 20-25 days p.i.), pigs will be sacrificed and sera will be collected, tested, distributed in aliquots and lyophilised.

1.1.2 To establish a *Trichinella*-positive standard pig serum

The EURLP aims at establishing a collaboration with the Institute for Reference Material and Measurements (IRMM), Joint Research Centre of the European Commission, to develop an international standard *Trichinella*-positive pig serum. The potential reference material, already available at the EURLP, needs to be further characterized with respect to several parameters of relevance. Indeed, if the reference material is to be used as an arbitrary standard, it must be ensured that its production is reproducible, i.e. the parameters to be reproducible and characterized, must be known. Thus, the ELISA target compounds would have to be quantified in the candidate reference material through collaborative studies.

1.1.3 To increase and maintain the serum bank of *Trichinella*-infected humans

Serum samples and/or blood spots will be collected from infected people during trichinellosis outbreaks occurring in different European countries or outside Europe. Serum samples from people with a confirmed diagnosis of trichinellosis will be tested by ELISA and western blot, distributed in aliquots, lyophilised and stored at +4°C. The database of this serum bank will be updated accordingly.

1.1.4 To produce reference *Trichinella* antigens for serology

Excretory/secretory (E/S) antigens will be produced from *Trichinella* spp. larvae in order to supply NRLs with the reference antigens for diagnostic purposes.

1.1.5 Maintenance of reference strains of *Trichinella in vivo*

Reference strains for each species or genotype of *Trichinella* identified so far will be maintained in laboratory animals. Fresh mouse carcasses infected with *Trichinella* species/genotypes will be provided to laboratories for training and for typing of wild isolates. *Trichinella* spp. larvae from reference strains will be stored in ethyl alcohol and forwarded to laboratories as reference material. To increase the quality and the reliability of the maintenance of these reference strains *in vivo*, the identity of each infected mouse will be monitored by a microchip inserted under the mouse skin.

1.1.6 Screening of commercial kits to detect anti-*Trichinella* IgG in pig sera

According to the Commission Regulation (EC) 2075/2005, a serological test may be used once a suitable test is validated by the EURLP to monitor the circulation of *Trichinella* infections in pig herds. The ELISA test has been validated by the EURLP and can be used to monitor the circulation of *Trichinella* parasites in pig herds. A plethora of commercial kits to detect anti-*Trichinella* IgG in swine are now commercially available, but none of them has been validated by the EURLP. Since one of the core duties of the EURLP is to give critical advices, we plan to invite the companies producing ELISA kits for the detection of anti-*Trichinella* IgG in swine sera to provide us with their kits in order to determine their performance and, in particular, their sensitivity, specificity, inter- and intra-assay variation, reproducibility and robustness, using a panel of known pig sera with different levels of IgG. The results will be communicated by a publication on a peer-reviewed international journal.

1.1.7 Diagnostic activity with accredited methods

Diagnostic samples provided by NRLs will be tested with the following accredited tests:

i. Identification of anti-*Trichinella* IgG antibodies in swine sera
ii. Identification of anti-*Trichinella* IgG antibodies in human sera
iii. Detection of *Trichinella* larvae in meat samples
iv. Identification of parasites of the genus *Trichinella* by a multiplex-PCR analysis.

1.2 Anisakidae worms

1.2.1 To increase and maintain the collection of Anisakidae worms and/or their genomic DNAs

Reference larvae will be collected directly from naturally infected fish; the DNA will be extracted and stored. Alternatively, reference larvae will be requested to European and extra-European laboratories with specific background. The database of this genetic bank will be updated accordingly.

1.2.2 Diagnostic activity with the accredited method

Anisakidae worms isolated from fish products by NRLs will be identified using the accredited PCR-RFLP test.

1.3 Echinococcus

1.3.1 To maintain and improve the genetic bank of the genus *Echinococcus*

Adult, larval and egg stages will be collected from different species of final and intermediate hosts originating from different geographical regions. The DNA will be extracted and stored at -20°C. The database of this genetic bank will be updated accordingly.

1.3.2 To establish a serum bank of *Echinococcus*-infected humans

Serum samples from *E. granulosus* and *E. multilocularis* infected humans with a confirmed diagnosis will be collected, aliquoted and stored at -80°C. The database of this serum bank will be updated accordingly.

1.3.3 Diagnostic activity with the accredited method

Echinococcus granulosus larvae, adult worms or eggs detected in intermediate and final hosts by NRLs, will be analysed using the accredited PCR method for the identification at the species/genotype level.

1.4 Other Cestodes

1.4.1 To maintain and improve the genetic bank of zoonotic cestodes such as those of the genus *Taenia* and *Diphyllobotrium*

Adult, larval and egg stages of zoonotic cestodes will be collected from infected hosts, both humans and animals. Genomic DNA will be extracted and stored. The DNA will be also identified by an available molecular test and the obtained sequences will be compared with those present in GeneBank. The database of this genetic bank will be updated accordingly.

1.5 Opisthorchidae trematodes

1.5.1 To maintain and improve the genetic bank of zoonotic Opisthorchidae trematodes

Adult, larval and egg stages of trematodes of the family Opisthorchidae will be collected from final and intermediate hosts, both humans and animals. Genomic DNA will be extracted and stored. The DNA will be also identified by an available molecular test and the obtained sequences will be compared with those present in GeneBank. The database of this genetic bank will be updated accordingly.

1.5.2 To maintain and improve the serum bank of *Opisthorchis*-infected humans

Serum samples from *Opisthorchis* spp. infected humans with a confirmed diagnosis will be collected, aliquoted and stored at -80°C. The database of this serum bank will be updated accordingly.

1.6 *Cryptosporidium*

1.6.1 To maintain and improve the genetic bank of protozoa of the genus *Cryptosporidium*

Cryptosporidium spp. oocysts will be collected from domestic and wild animals, humans and environmental samples. Nucleic acids will be extracted and stored at -20°C until their identification by molecular tools. The database of this genetic bank will be updated accordingly.

1.7 *Giardia*

1.7.1 To maintain and improve the genetic bank of protozoa of the genus *Giardia*

Giardia spp. cysts will be collected from domestic and wild animals, humans and environmental samples. Nucleic acids will be extracted and stored at -20°C until their identification by molecular tools. The database of this genetic bank will be updated accordingly.

2 Research

2.1 Barcoding of zoonotic and non zoonotic helminths and protozoa parasitizing domestic animals and foodstuffs (multi years)

The use of short DNA sequences as a barcode to differentiate taxa and to discover new species, is becoming a popular technique in the scientific community. There are many possible applications of DNA barcoding, from biodiversity studies to food tracking. Our task will be the identification of specific DNA regions that could be used for the identification at the species, genus or family level and the evaluation of their potential for a large scale application. In the field of food-borne parasites, we would like to focus our attention on: 1) the liver flukes circulating in freshwater fish in Europe. *Opisthorchis felineus* is the main zoonotic species, but other species can be occasionally zoonotic such as *Metorchis bilis* and *Echinostoma* sp.; their larval stage (the metacercaria) present in fish and the eggs shed by the definitive hosts, cannot be easily distinguished from the metacercariae of non-zoonotic liver flukes; 2) nematode larvae resembling *Trichinella* that are often collected during the digestion of muscle samples will be also identified at the species, genus or family level; and 3) cestode cysts detected in livestock tissues to distinguish between those belonging to zoonotic parasites from those which do not infect the human beings.

2.2 Identification of *Toxoplasma gondii* proteins specific for the oocyst stage

We plan to validate the efficacy of a novel monoclonal antibody (anti-TgOWP3 mAb 3B11) and of other mAbs possibly produced in the second semester of 2011, in the coprodiagnosis and in the immunomagnetic capture of *T. gondii* oocysts in faecal and/or environmental samples. In addition, we would like to pursue a second objective. Currently available serologic assays can establish if an individual has been exposed to *T. gondii*, but are not able to discriminate between the two parasite developmental stages which are responsible for virtually all *Toxoplasma* human infections, i.e., the environmentally resistant oocysts or the viable tissue cysts contained in undercooked or raw meat. Taking advantage from the identification in our laboratory of several novel proteins expressed by the *T. gondii* oocyst/sporozoite, we intend to assess the immunogenic potential of these putative stage-specific molecules in natural infections, with the final goal to develop a serologic test able to identify oocyst-driven disease transmission. The serodiagnostic value of the novel proteins will be assessed through ELISA assays, using recombinant versions of the oocyst/sporozoite molecules and a panel of *Toxoplasma*-positive sera derived from human subjects or experimentally-infected animals exposed to known parasite infective stages.

2.3 Study of the genetic structure of *Trichinella spiralis* and *Trichinella britovi*

The genetic structure of *T. spiralis* isolates circulating within a restricted geographical areas (e.g. Extremadura region, Spain) will be investigated by the analysis of the microsatellite variability. In the last years, approximately 50 isolates were collected from wild boars of Extremadura. The muscle larvae will be genotyped using the 18 genetic markers isolated to date. The search for microsatellites will continue using the collection of DNA supercontig from the whole genomic sequencing (WGS) project now available on public databases. The use of genetic markers isolated from the genome of *T. spiralis* has proven to be ineffective in the study of the genetic variability of *T. britovi*. For this reason, we plan to develop a specific *T. britovi* WGS project. The project will include: 1. Circularization-Ready Fragments. Genomic DNA

will be sheared into 20 Kb, 8 Kb or 3 Kb fragments and adaptors will be added to the end of each fragment; 2. Circularized DNA. DNA will be circularized; 3. Paired Ends Library Construct. The circularized DNA will be fragmented and fragments containing the added adaptors will be isolated and amplified for sequencing; 4. Paired Ends Library. The resulting library will consist of true paired end reads with two end tags averaging over 150 bp and separated by 20 Kb, 8 Kb or 3 Kb; and 5. Assembly. Assembly of sequences in supercontigs.

2.4 Identification of *Trichinella*-specific antigens most frequently recognised by swine sera by Western blot

A major problem in the serological diagnosis of parasitic infections is the cross-reactivity with parasitic and non-parasitic antigens. Crude and excretory/ secretory antigens (ESA) from muscle larvae are widely used for diagnosis, but, since these antigens are complex mixtures of molecules, cross-reactivity to other antigenically related parasites may occur. In fact, the presence of shared antigens of *Trichinella* sp. has been largely reported in other parasites and pathogens and, even if several serological techniques have been developed and attempts have been made to increase the specificity of the available tests, there is still a high percentage of false-positive reactions. In a previous study, it was shown that the specificity of ELISA is greatly influenced by the panel of sera tested, increasing the percentage of false positive-reaction when considering swine with other parasitic and non-parasitic infections or with non-infective pathologies. The aim of this study is to determine in detail the distinctive pattern of reactivity of the *Trichinella* positive swine sera with ESA by Western blot to discriminate the non specific-cross reactive bands from those corresponding to *Trichinella*-specific antigens. To this end, we will test swine sera from *Trichinella* infected swine and from swine with other parasitic or non parasitic infections, or swine coming from highly endemic areas for parasitic infections. This study will allow the establishment of a model of sera reactivity with *Trichinella* ESA by Western blot which will be used to confirm ELISA positive sera.

2.5 Molecular identification and characterization of excretory/secretory antigens from muscle larvae

A major problem in the serological diagnosis of nematode infections in animals and humans is the cross-reactivity with other parasitic and non-parasitic antigens. Crude and excretory/secretory antigens (ESA) from *Trichinella spiralis* muscle larvae are widely used for diagnosis but, since these antigens are complex mixtures of molecules, cross-reactivity to other antigenically related parasites may occur. Using immunoblot, a limited set of bands in the ESA have been now proved to be recognised in 100% of the sera from naturally or experimentally *Trichinella*-infected pigs. As part of the collaboration program between EURLP and the Institute for Reference Material and Measurements (IRMM), to develop an international standard *Trichinella*-positive pig serum a proteomic approach will be used for the identification and characterization at the molecular level of ESA from muscle larvae. Taking advantage from the recently sequenced *Trichinella spiralis* genome, a mass spectrometry technique will be used. ESA molecules will be immunoprecipitated by Ig present in the temporary reference positive pig sera (or, as control, form temporary reference negative pig sera). Immunoprecipitated materials will be separated on SDS-PAGE and subjected to analysis with nanoflow reversed-phase liquid chromatography tandem mass spectrometry (RP-LC-MS/MS). The genes coding for the identified proteins will be then cloned and expressed in bacterial or

yeast background and tested by ELISA for their specific reactivity against a large panel of positive and negative pig and human sera. The antigenic portions of the selected protein will be further characterized by PEP-SPOT analysis to allow the synthesis of standardized *Trichinella*-specific antigens for multiple diagnostic tests.

2.6 Study of the genetic polymorphisms of *Echinococcus granulosus* sensu lato

The polymorphism exhibited by nuclear and mitochondrial markers conventionally used for the genotyping of different parasite species and strains does not reach the level necessary for the identification of genetic variants in *E. granulosus* s.l. linked to restricted geographical areas. EmsB is a tandemly repeated multilocus microsatellite that proved its usefulness for the study of genetic polymorphisms within the species *E. multilocularis*. In the present study, EmsB will be used to characterize *E. granulosus* sensu lato samples collected from different host species originating from Eastern Europe (Bulgaria, Romania, Hungary). The conventional mitochondrial cox1 and nad1 markers identified genotypes G1, G2, G3, G4, G5, G6, and G7, which are clustered into four groups corresponding to the species *E. granulosus* sensu stricto, *E. equinus*, *E. ortleppi*, and *E. canadensis*. With the same samples, EmsB provided a higher degree of genetic discrimination and identified variations that correlated with the relatively small-scale geographic origins of the samples. In summary, the EmsB microsatellite exhibits an interesting potential for the elaboration of a detailed map of the distribution of genetic variants and therefore for the determination and tracking of the source of cystic echinococcosis.

2.7 Development and validation of a molecular test to identify parasites of the genus *Opisthorchis* (multi-years)

The food-borne parasite *Opisthorchis felineus* was recently identified as source of epidemics linked to raw fish consumption and whose real spread in Europe is still little known. The morphological identification of *O. felineus* adults is often difficult and requires considerable experience while the identification of eggs or larvae (metacercaria) is restricted to specialists. Hence the need for a molecular based identification system allowing a reliable and quick response and that can be used also by non-specialist staff. Our goal is to set-up a rapid molecular test able to identify *O. felineus* eggs in the faeces of infected patients or in natural final hosts (e.g. cats, dogs, foxes), metacercariae in fish muscle tissues, and adults collected from necropsies of final natural hosts. The initial task will be the identification of specific DNA regions that could be used for the identification of the parasite, then specific primers will be designated to amplify the target DNA region by a PCR protocol. The method will be validated by testing different *O. felineus* isolates including the three life stages (i.e. adults, eggs and metacercariae) using reference material.

2.8 Development and validation of a serological tests to detect anti-*Opisthorchis* spp. IgG in human sera (multi-years)

Excretory/secretory antigens (ESA) to be used in an ELISA will be produced by in vitro cultivation of adult flukes which will be obtained from hamsters experimentally infected by ingestion of metacercariae collected from naturally infected freshwater fish. Livers will be tacked out from the hamsters and alive adult worms will be collected under sterile conditions. The worms will be washed with cold sterile phosphate-buffered saline (PBS) containing antibiotics. Two-three adult liver flukes/mL will be maintained in Dulbecco medium for 20 hrs in an atmosphere of 5%

CO_2 at 37°C. Further, the medium will be centrifuged for 10 min at 1000 rpm to remove the dead worms and debris. Alive worms will be put again in fresh culture medium and maintained for other 20 hours. The supernatant will be further centrifuged for 30 min at 10000 rpm and filtered with a syringe-driven 0.45-µm filter unit and the protein contain will be determined. Serum samples and/or blood spots will be collected from infected people during opisthorchiasis outbreaks occurring in different European countries. Serum samples from people with a confirmed diagnosis of opisthorchiasis will be tested by ELISA, distributed in aliquots, lyophilized and stored at +4°C. An indirect ELISA will be set up and the working dilutions of ESA, conjugate and sera will be by determined by checkerboard titration using as positive control sera from infected people.

2.9 Development of molecular test to identify *Dientamoeba fragilis* in human and animal faecal samples (two-years)

Dientamoeba fragilis is a protozoan parasite found in the gastrointestinal tract of humans, and is currently classified as a flagellate, although it lacks a flagellum. The organism has a worldwide distribution and the prevalence of *D. fragilis* in humans varies widely from 0.3% to 52%. In contrast to other pathogenic protozoa which have high prevalence in developing regions of the world, *D. fragilis* is also prevalent in industrialized countries. Apart from its evident association with humans, few reports have suggested the presence of *D. fragilis* in animal hosts. Indeed, the host range of this parasite remains to be determined. Moreover, very little is known on the transmission route(s) of this parasite. Diagnosis of *D. fragilis* has traditionally relied upon microscopy of fixed fecal smears. Because of the “fragile” nature of the organism, prompt fixation of clinical specimens is essential as the trophozoites degenerate rapidly once passed in stool samples. Diagnosis is improved by the use of PCR-based techniques, and in particular, real-time PCR protocols show excellent specificity and sensitivity when applied to human fecal samples, and can be considered as the gold standard in the diagnosis of dientamoebiasis. Recently, we discovered this parasite in farmed swine and in persons working at the farm, suggesting a possible transmission of this parasite from pigs to humans, i.e. a possible new zoonosis linked with swine. We intend to compare two real-time assays that target the 18S and the 5.8S genes in the ribosomal cluster, in order to evaluate their performance on both human and animal isolates available in the genetic banks of our laboratory. This could also provide information on the circulation of the parasite in non-human hosts.

2.10 Development of analytical methods for the speciation of parasites of the genus *Taenia*

Taenia is a relatively large genus containing approximately 42 valid species and 3 subspecies. *Taenia solium*, *Taenia saginata*, *Taenia asiatica* and *Taenia multiceps* are all *Taenia* tape worms which cause human infections, using either cattle or pigs or sheep as intermediate hosts. DNA will be extracted from adult worms or larvae. After purification, DNA samples will be stored at −20°. The PCR amplification will be performed for five mitochondrial genes: A (nad1, forward 5′-CARTTTCGTAAGGGB CCWAAWAAGGT, reverse 5′-CCAATTTCYTGAAGTTAACAGCATCA), B (rrnS, forward 5′- AGGGGATAGGRCACAGTGCCAGCATCTGCGG, reverse 5′- AATTCA TTTAAAGTTACCTTGTTACGACTTACCTC), C (nad5, forward 5′- TATATGAGTT AGTTTTAAGCATTAATTATGG, reverse 5′- GGAAAHCTAGCACTCTTDGTAA), D (COI, forward 5′- TTGAATTTGCCACGTTTGAATGC, reverse 5′- GAACCTAACGAC

ATAACATAATGA). Forward and reverse sequencing will be carried out on PCR products by a capillary DNA sequencer. Reference sequences will be achieved using GenBank with the BLAST system; forward and reverse sequences will be aligned and compared using Accelsys gene 2.5 program. Molecular markers will be used to point out the genetic diversity within and between the species. PCR products will be sequenced on both strands and the sequences will be compared with all available sequences using BLAST. The genetic diversity of the five molecular markers within and between species will be determined.

3 Interlaboratory comparison studies

According to the requests of NRLs expressed in the course of the sixth NRL workshop, held in Rome from 23 to 24 May, 2011, three proficiency tests (PTs) will be organised by the EURLP in the course of 2011.

3.1 Proficiency test (PT) to detect *Trichinella* larvae in meat samples

The sixth PT on the detection of *Trichinella* larvae in meat samples, will be organised among NRLs to evaluate the sensitivity of the magnetic stirred method as reported in the EU legislation 2075/2005 on *Trichinella*. Test samples (100 or 35 g meatballs made with diaphragm tissue from pigs, horses and/or wild boars) will be spiked with a known number of *T. spiralis* larvae obtained from experimentally infected mice. Each NRL will receive five samples containing three different numbers of *Trichinella* larvae, plus a negative control sample. Samples will be packed and sent as bio-hazardous material in cool freeze containers to ensure a stable temperature. Every participating partner in the proficiency test will be notified in advance about the timetable and when to receive the test panels along with the protocol. The test results from each laboratory will be evaluated, compared to those of the previous years, and possible critical points will be identified and corrected.

3.2 Proficiency test to identify *Trichinella* larvae at the species level by a PCR-derived method

The PT will be organised among NRLs to evaluate their skill to properly identify *Trichinella* larvae at the species level. *Trichinella* larvae from reference strains representing the species circulating in Europe and those which have been occasinally imported from non-EU countries into Europe, will be collected from infected mice by artificial digestion and stored in 0.5 ml conical vials with ethyl alcohol. Vials will be coded and forwarded to participating labs for molecular identification by a PCR derived method according to the PCR- method used in each laboratory. Participant laboratories will be invited to identify single larvae instead of a pool of larvae.

3.3 Proficiency test to identify *Echinococcus* sp. adult worms in the intestinal content of a definitive host

For the third time, this PT will be organised among NRLs to detect adult worms or their portions of *Echinococcus* sp. spiked in the natural matrix (intestinal content). Each NRL will receive five samples containing three similar amounts of worms, plus two negative controls. Samples will be packed and sent as bio-hazardous material in cool freeze containers to ensure a stable temperature. Every participating partner in the PT will be coded (lab code) and notified in advance about the timetable and when to receive the test panels along with the protocol. The test results from each

laboratory will be evaluated, compared to those of the previous years, and possible critical points will be identified and corrected.

4 Workshop

In the first half of 2012, a two day-workshop will be held at the Istituto Superiore di Sanità of Rome, or in another MS, to present and discuss the results of the PTs and other issues including epidemiological problems related to foodborne parasitic zoonoses occurring in the MS. Some experts in the field of foodborne parasitic zoonoses will be invited to present the most recent knowledge on the epidemiology, diagnosis and control of these pathogens.

5 Visit to NRLs

Qualified personnel of the EURLP will visit two NRLs to assist them as required by circumstances. The selection of the NRLs will be done with an agreement among NRL, EURLP and the Commission. The outcome of the visits will be reported to the Commission.

6 Training for the personnel of NRL and developing countries

On request by NRLs within EU or by governmental institutions of developing countries, personnel will be hosted at the EURLP to be trained on different detection methods of foodborne parasites and quality control systems.

7 Further development and up date of the web site of the EURL for parasites

To improve the usefulness of the EURLP web site, contents within the *Forum* section will be fully accessible, whereas only authorized users will be able to post documents on it. In addition, the section will be updated by uploading fact sheets on the most important foodborne parasitic diseases, describing the parasite life cycles, their epidemiology in European countries, their pathogenicity in humans, diagnostic methods, etc.

8 Standardization of methods for the detection of parasites in food

A EURLP representative will participate to the next ISO/TC 34/SC 9 and CEN/TC 275/WG 6 joint meetings to be held in Belgium in June, 2012, in order to follow as project leader of TAG 7 the standardization process on *Trichinella*. Moreover, the CEN/TC 275/WG 6 has decided at the last meeting held in Bournemouth (resolution 264) to continue the standardization work on *Trichinella* at ISO level in a sub-group of ISO/TC34/SC9/WG6, with the participation of the EURLP representative.

9 Development of reference materials for diagnostic methods to detect *Trichinella* infections

A collaboration between EURLP and Institute for Reference Material and Measurements (IRMM) Joint Research Centre of the European Commission (Belgium) have started with a double aim: 1.- to carry out stability studies of *Trichinella* positive swine sera already stored at the EURLP based on the isochronous sample storage schemes applied by IRMM for the characterization of

reference materials. Stability will be studied at short and a long term, the former is already concluded, the latter is in progress; 2.- To develop a certified reference material based on the reproducible excretory/secretory antigen preparation which will be the basis for standardisation. To this end two options will be investigated in parallel: a) protocol defining the antigen production (collection and preparation of E/S antigens (at EURLP) based on 1) systematic investigation on the reproducibility of E/S antigen preparation and refinement preparation protocols (at EURLP); 2) systematic studies on the reproducibility of binding the antigens to the micro titre plates (at IRMM and at EURLP); b) definition and selection of significant measurands: 1) attempts to identify the various antigens in the E/S extracts (at EURLP) by SDS PAGE and western blotting of *Trichinella* positive swine sera (at EURLP); 2) two dimensional electrophoresis (at IRMM); 3) mass spectrometry and peptide analysis after enzymatic digest (TOF, LC-MS, LC-MS-MS) (at IRMM).

10 Validation of apparatuses for the detection of *Trichinella* larvae in meat samples

According to the Guidelines for the validation of apparatuses for the detection of *Trichinella* larvae in meat samples by digestion, the EURLP will organize the validation process involving four National Reference Laboratories for Parasites. When the "Guidelines on the requirements of serological kits for the detection of anti-*Trichinella* IgG in pig sera to be used in monitoring programs", will be approved by the DG SANCO, commercial kits will be validated according to the company requests.

11 Quality assurance system

The continuous improvement of the EURLP Quality Assurance System, is a key factor to assure to the NRLs the highest level of reliability of EURLP services. For this aim, the EURLP will apply for an additional accreditation according to the ISO 17043:2010 standard as a Proficiency Test provider. This accreditation will be probably reached in 2012, as soon as the Italian accreditation body, ACCREDIA, will be able to grant such accreditation.

12 Meeting at the DG SANCO

The Director of EURL for Parasites will attend the yearly meeting at the DG SANCO.

Rome, 23th August, 2011

The Director of EURL for Parasites
Dr. Edoardo Pozio