EUROPEAN UNION REFERENCE LABORATORY FOR PARASITES

WORK PROGRAMME 2014

The 2014 working programme of EURL for Parasites (EURLP) consists of the following activities:

1. Production of reference material and diagnostic support to the NRLs and developing countries

1.1 *Trichinella*

1.1.1 To increase and maintain the 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 and Western blot, distributed in aliquots, lyophilised and stored at +4°C. The database of the serum bank will be updated accordingly.

Objectives:	*Availability of* Trichinella*-positive pig sera for the validation of serological tests*
Expected outputs:	*A statistically significant number of well characterized pig sera*
Performance indicators:	*Increase of the available pig sera*

1.1.2 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 the validated ELISA and Western blot, distributed in aliquots, lyophilised and stored at +4°C. The database of this serum bank will be updated accordingly.

Objectives:	*Availability of* Trichinella*-positive human sera for the validation of serological tests*
Expected outputs:	*A statistically significant number of well characterized human sera*
Performance indicators:	*Increase of the available human sera*

1.1.3 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.

Objectives:	*Supply NRLs, labs in developing countries and EURLP with* Trichinella *ES antigens*
Expected outputs:	*Production of* Trichinella *ES antigens*
Performance indicators:	*Number of milligrams of produced* Trichinella *ES antigens*

1.1.4 Maintenance of *Trichinella* reference strains *in vivo*

Reference strains for each of the nine species and three genotypes 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 as reference material for typing new isolates. *Trichinella* spp. larvae from reference strains will be stored in ethyl alcohol and forwarded to laboratories as reference material.

Objectives:	*Further development of the Bio-bank of* Trichinella *parasites for European, extraeuropean, and international institutions*
Expected outputs:	*Production of reference material*
Performance indicators:	*Number of* Trichinella *species and genotypes maintained in vivo*

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

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 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 pig sera with known different levels of IgG.

Objectives:	*Availability on the EU market of reliable commercial kits*
Expected outputs:	*Increased diagnostic quality*
Performance indicators:	*Number of evaluated kits*

1.1.6 Diagnostic activity with accredited methods

Diagnostic samples provided by NRLs or third countries will be tested with the following accredited tests (www.accredia.it/accredia_labsearch.jsp?ID_LINK =293& area=7&&):

i. Identification of anti-*Trichinella* IgG antibodies in swine sera (MI-01 rev. 5, 2009)
ii. Identification of anti-*Trichinella* IgG antibodies in human sera (MI-03 rev. 2, 2009)
iii. Detection of *Trichinella* larvae in meat samples by digestion (EC 2075/2005)
iv. Identification of parasites of the genus *Trichinella* by a multiplex-PCR analysis (MI-02 rev. 4, 2009).

Objectives:	*Diagnostic support to NRLs and developing countries*
Expected outputs:	*Confirmatory diagnoses*
Performance indicators:	*Number of tested samples*

1.2 Anisakidae

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

Reference larvae will be collected from naturally infected fish; the DNA will be extracted and stored at -20°C. Alternatively, reference larvae will be requested to European and extra-European laboratories having an expertise in this subject. The database of this collection will be updated accordingly.

Objectives:	*Development of a genetic-bank of* Anisakidae *parasites for European, extraeuropean, and international institutions*
Expected outputs:	*Supply of reference material*
Performance indicators:	*Number of* Anisakidae *worms characterized and stored*

1.2.2 Diagnostic activity with the accredited method

Anisakidae worms isolated from fish products by NRLs and third countries will be identified using the accredited PCR-RFLP test (www.accredia.it/accredia_labsearch.jsp?ID_LINK =293& area=7&&):

i. Identification at species level of parasites of the family Anisakidae by PCR/RFLP (MI-04 rev. 1, 2010)

Objectives:	*Diagnostic support to NRLs and developing countries*
Expected outputs:	*Confirmatory diagnoses*
Performance indicators:	*Number of tested samples*

1.3 *Echinococcus*

1.3.1 To increase and maintain 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.

Objectives:	*Further development of a genetic bank of* Echinococcus *parasites for European, and non-European institutions*
Expected outputs:	*Production of reference material*
Performance indicators:	*Number of* Echinococcus *isolates characterized and stored*

1.3.2 To increase and maintain the 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.

Objectives:	*Availability of* Echinococcus*-positive human sera for the validation of serological tests*
Expected outputs:	*A statistically significant number of well characterized human sera*
Performance indicators:	*Increase of the available human sera*

1.3.3 Diagnostic activity with the accredited method

Echinococcus granulosus larvae, adult worms or eggs detected in intermediate and final hosts by NRLs and third countries, will be identified using the accredited method (www.accredia.it/accredia_labse arch.jsp?ID_LINK =293& area=7&&):

i. Identification of *Echinococcus granulosus* complex at genotype/species level by PCR and sequencing (MI-05 rev. 1, 2010)

Objectives:	*Diagnostic support to NRLs and developing countries*
Expected outputs:	*Confirmatory diagnoses*
Performance indicators:	*Number of tested samples*

1.4 Other Cestodes

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

Adult, larval and egg stages of zoonotic cestodes not belonging to the genus *Echinococcus* will be collected from infected hosts, both humans and animals. Genomic DNA will be extracted and stored. The DNA will be amplified by PCR and the amplicons will be sequenced. The obtained sequences will be compared with those present in GenBank. The database of this genetic bank will be updated accordingly.

Objectives:	*Development of a genetic bank of Cestode parasites for European, and non-*

	European institutions
Expected outputs:	*Production of reference material*
Performance indicators:	*Number of Cestode worms characterized and stored*

1.5 Trematodes

1.5.1 To increase and maintain the genetic bank of zoonotic trematodes of the Opisthorchidae family

Adult, larval and egg stages of trematodes of the family Opisthorchidae will be collected from final, both humans and animals, and intermediate hosts. Genomic DNA will be extracted and stored. The database of this genetic bank will be updated accordingly.

Objectives:	*Development of a genetic-bank of Opisthorchidae parasites for European, and non-European institutions*
Expected outputs:	*Production of reference material*
Performance indicators:	*Number of Opisthorchidae worms characterized and stored*

1.5.2 To increase and maintain 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.

Objectives:	*Availability of* Opisthorchis*-positive human sera for the validation of serological tests*
Expected outputs:	*A statistically significant number of well characterized human sera*
Performance indicators:	*Increase of the available human sera*

1.5.3 To produce reference *Opisthorchis* antigens for serology

Excretory/secretory (E/S) antigens will be produced from *Opisthorchis felineus* adult worms for the in-house serodiagnosis and to supply NRLs and third countries with the reference antigens for diagnostic purposes.

Objectives:	*Supply NRLs, labs in developing countries and EURLP with* Opisthorchis *ES antigens*
Expected outputs:	*Production of* Opisthorchis *ES antigens*
Performance indicators:	*Number of milligrams of produced* Opisthorchis *ES antigens*

1.5.4 Diagnostic activity with accredited methods

Diagnostic samples provided by NRLs or third countries will be tested with the following accredited tests (www.accredia.it/accredia_labsearch.jsp?ID_LINK =293&area=7&&):

i. Detection of anti-*Opisthorchis* antibodies in human serum by indirect ELISA (MI-07 rev 0, 2012)

ii. Identification of *Opisthorchis* sp. by PCR (MI-08 rev 0, 2012)

Objectives:	*Diagnostic support to NRLs and developing countries*
Expected outputs:	*Confirmatory diagnoses*
Performance indicators:	*Number of tested samples*

1.6 *Cryptosporidium*

1.6.1 To increase and maintain 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.

Objectives:	*Further development of a genetic bank of* Cryptosporidium *parasites for European, and non-European institutions*
Expected outputs:	*Production of reference material*
Performance indicators:	*Number of* Cryptosporidium *isolates characterized and stored*

1.6.2 Diagnostic activity with the accredited method

Diagnostic samples provided by NRLs or third countries will be tested with the following accredited test (www.accredia.it/accredia_labsearch.jsp?ID_LINK=293& area =7&&):

i. Identification at the species level of oocysts of *Cryptosporidium* spp. by PCR/RFLP (MI-06 rev 1, 2011)

Objectives:	*Diagnostic support to NRLs and developing countries*
Expected outputs:	*Confirmatory diagnoses*
Performance indicators:	*Number of tested samples*

1.7 *Giardia*

1.7.1 To increase and maintain 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. The database of this genetic bank will be updated accordingly.

Objectives:	*Development of a genetic bank of* Giardia *parasites for European and non-European institutions*
Expected outputs:	*Production of reference material*
Performance indicators:	*Number of* Giardia *isolates characterized and stored*

1.7.2 Diagnostic activity with the accredited method

Diagnostic samples provided by NRLs or third countries will be tested with the following accredited test (www.accredia.it/accredia_labsearch.jsp?ID_LINK =293&area=7&&):

i. Identification at the assemblage level of cysts of *Giardia duodenalis* by PCR/RFLP (MI-09 rev 0, 2012)

Objectives:	*Diagnostic support to NRLs and developing countries*
Expected outputs:	*Confirmatory diagnoses*
Performance indicators:	*Number of tested samples*

1.8 *Toxoplasma gondii*

1.8.1 To increase and maintain the genetic bank of *T. gondii* isolates

A panel of *Toxoplasma gondii* strains will be produced and implemented over time. The strain collection will include tachyzoites belonging to either of the three major genotypes, denominated I, II and III, which account for approximately 95% of *T. gondii* strains circulating in Europe and North America. In addition, parasite isolates of the so-called "atypical" genotypes will be collected. On request, viable tachyzoites, genomic DNA or tachyzoite protein lysates of any given strain will be supplied to European laboratories.

Objectives:	*Development of a genetic bank of* T. gondii *parasites for European, and non-European institutions*
Expected outputs:	*Production of reference material*
Performance indicators:	*Number of* T. gondii *isolates characterized and stored*

2 Ongoing activities towards the development of new diagnostic tools

2.1 Barcoding of zoonotic and non zoonotic helminths and protozoa parasitizing domestic animals and foodstuffs

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 will continue to focus on: 1) the liver flukes circulating in freshwater fish in Europe; 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 humans.

Objectives:	*Diagnostic support to NRLs and developing countries*
Expected outputs:	*Identification of 'unusual' foodborne parasites*
Performance indicators:	*Number of tested samples*

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

Validated oocyst/sporozoite-specific proteins able to induce a humoral immune response in experimentally infected pigs will be employed to screen a panel of human sera. To this aim, we will collect sera from various groups of individuals, including negative subjects, chronically infected subjects and acutely infected women. This panel of sera will be tested by western blot using one or more validated antigens. The major implication of this survey will be the evaluation of the prevalence of oocyst-derived *T. gondii* infections and in particular the evaluation of the risk of pregnant women to be infected by this parasite stage in the perinatal period.

Objectives:	*Development of a diagnostic test to distinguish human infection caused by cysts or oocysts ingestion*
Expected outputs:	*Production of MAb specific for* Toxoplasma *oocyst wall proteins*
Performance indicators:	*Number of MAbs produced*

2.3 Bioassay for *Toxoplasma gondii* bradyzoites/tachyzoites in mice

The role of some livestock species (e.g. cattle) as source of infection for humans is still under discussion. In fact, the detection of anti-*T. gondii* antibodies in animals does not imply the presence of viable and infective *T. gondii* bradyzoites/tachyzoites in animal tissues. It follows that a bioassay is the only way to answer this question. Tissue samples, mainly from the heart, will be homogenized in saline and digested in a pepsin-HCl solution. The digest will be filtered, concentrated in saline containing antibiotics and the final suspension will be inoculated subcutaneously into mice. Serum samples will be collected from mice before and 30 days after the inoculum and tested by a modified agglutination test to detect the seroconversion. This method will allow also the isolation of *T. gondii* isolates circulating in Europe and/or in livestock imported from third countries.

Objectives:	*Identification of livestock species at risk for* T. gondii *transmission to humans by the consumption of raw tissues*
Expected outputs:	*Isolation of* T. gondii parasites by a bioassay
Performance indicators:	*Number of muscle samples from livestock consumed in Europe, tested for the presence of* T. gondii *infectious cysts*

2.4 Development of a molecular test to identify *Dientamoeba fragilis* in human and animal faecal samples

Dientamoeba fragilis is a protozoan parasite found in the gastrointestinal tract of humans, and is currently classified as a flagellate. The organism has a worldwide distribution and the prevalence of *D. fragilis* in humans varies widely from 0.3% to 52%. 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. 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. To better characterize transmission routes and to study the potential correlation between parasite strains and symptoms in the host, specific and informative genetic markers are needed. We have generated novel markers corresponding to different genes (coding for peptidases, kinases, structural proteins), and have developed and tested PCR assays for their amplification. We intend to explore the utility of these markers for the establishment of a multi-locus genotyping scheme, that will be evaluated on human and pig samples positive for *D. fragilis*.

Objectives:	*Development of analytical methods for the identification of* Dientamoeba fragilis *in human and pig faeces*
Expected outputs:	*Identification of species-specific primers*
Performance indicators:	*Number of isolates identified*

2.5 The 'omic' project on *Anisakis pegreffii*

In the frame of the "omic" project, the genome of *Anisakis pegreffii* and possibly the transcriptome of the L3 stage will be sequenced and analysed. In fact, the consumption of fish infected with the L3 stage of Anisakidae, such as *A. simplex* and *A. pegreffii*, has been associated with allergic reactions in sensitized people due to parasite antigenic molecules secreted or present at the surface of the larvae which are resistant either to freezing and cooking. Despite the increasing relevance

of this phenomenon, the identification of these antigenic proteins is still limited by the lack of genome data of these parasites or other related anisakidae. The description of the genome of at least one member of the Anisakidae family will provide the necessary information for a high throughput analysis such as proteomic and immunoproteomic associated with a mass spectrometry analysis leading to the unambiguous identification of all the potentially relevant antigens. This information will help in future to develop effective therapeutic approaches and/or screening tests. Briefly, the genomic DNA of *A. pegreffii* will be extracted from a single L3. Similarly, the messenger RNA will be extracted from a pool of *A. pegreffii* L3, either directly after the picking from the fish or after 5 days of *in vitro* culture to allow the expression of the gene coding for potential allergens. The obtained highly quality materials will be sufficient for whole genome and transcriptome sequencing by a "next generation" approach (i.e., Illumina). The sequence readswill be analysed and assembled using specific software (i.e., Mira). Due to the unknown size of the genome (that could range from 50 megabases, as in the parasite nematode *Trichinella spiralis*, to 250 megabases as for *Ascaris suum*) further strategies could be necessary to improve the quality of the genome sequencing data, especially for the non-coding portion of the genome. The sequence of the transcripts will also help in the correct assembly of the genome.

Objectives:	*Acquisition of data for the development of diagnostic tools and for epidemiological investigations*
Expected outputs:	*Important basic information on the genome and transcriptome of* Anisakis pegreffii
Performance indicators:	*Genomic and trascriptomic data*

2.6 Hazard identification by antigen characterization for fish nematodes other than those of the *Anisakis* genus

Crude extract and excretory/secretory (ES) antigens from *A. pegreffii*, *Pseudoterranova* spp. and *Contracaecum* spp. will be prepared from worms harvested from infected fish and fish products by several cycles of homogeneization-sonication followed by extraction in phosphate buffered saline. Hyper-immune sera to the parasites antigens will be obtained in rabbits using standard procedures. The antigenic profiles of the parasite extracts will be analyzed by western blotting (WB). The allergenic capacity of the different selected parasite antigens will be determined in animal models. Animals will be intraperitoneally immunized with 2-5 living L3 larvae of *A. simplex ss* as well as the same number of larvae of *A. pegreffii*, *Pseudoterranova* spp. and *Contracaecum* spp. Animals will be re-infected at week 8th and an oral challenge with 5 mg of homologous crude extract will be given at week 11. Sera will be collected at appropriate intervals and the presence of specific antibodies as well as the class of antibodies will be evaluated by ELISA and WB. Lymphocyte proliferation and cytokine production will be evaluated according to published protocols. In parallel, mice will be orally immunized with the selected untreated or heat-treated antigens in presence of cholera toxin or other adjuvant. The evaluation of the immune responses will be carried out as above. To determine the contact between Anisakidae parasites and human beings, by the detection of specific IgE, a panel of sera collected from fish-eating people with history of allergic reactions after fish consumption, will be screened by WB using crude extracts and allergen-enriched fractions from the different Anisakidae parasites.

Objectives:	*Identification of allergens in fish nematodes of the genera* Anisakis, Pseudoterranova *and* Contracaecum
Expected outputs:	*Characterization of antigens*
Performance indicators:	*Number of identified and characterized allergens*

2.7 Identification of polymorphic microsatellites in *Trichinella spiralis* and *Trichinella britovi*

After enucleating the genetic structure of the *T. spiralis* population circulating in the Extremadura region of Spain, it will be important to know if the pattern displayed in this area is similar to those present in other geographical regions. For this reason, the genetic markers previously selected (Extremadura study) will be used to analyze the genetic structure of the *T. spiralis* population circulating in wildlife of other European regions. The results will be compared to the previous one in order to detect the differences in the allelic composition and to select geographical markers. In addition, the genomes of *T. britovi* and *T. spiralis* will be compared to reveal similarities/differences between the two species. Homologous sequences differing for short nucleotide repeats (microsatellites) will be selected to be used as genetic markers. For each putative marker, a PCR primer pair will be produced to be used for the analysis of a panel of single larvae of *T. britovi* and *T. spiralis* in order to estimate the allelic polymorphisms. When polymorphic microsatellite markers will be available, the allelic structure of a large panel of *T. britovi* isolates circulating across Europe, will be investigated in order to highlight the geographical distribution of markers useful for monitoring.

Objectives:	*Development of a specific* Trichinella britovi and T. spiralis *WGS to study the genetic variability of this species*
Expected outputs:	*Production of genetic markers specific for* Trichinella britovi *and* T. spiralis
Performance indicators:	*Number of genetic markers produced*

2.8 Population study of *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis*

Echinococcus granulosus sensu lato is a complex of species causing cystic echinococcosis (CE). Recent phylogenetic studies based on both mitochondrial and nuclear DNA genes have revealed that *E. granulosus* sensu lato consists of at least four species. Among them, *E. granulosus* sensu stricto is known to have a broad geographical distribution and a wide host range. The three known genotypes of *E. granulosus* s.s. are called the sheep (G1), the Tasmanian sheep (G2) and the buffalo (G3) genotypes. The main purpose of this study will be to characterize the population genetic structures of the European isolates of *E. granulosus* sensu stricto by the microvariant analysis. The knowledge derived from this analysis will be useful for developing new molecular-based tools, with improved specificity and sensitivity, for the diagnosis of this parasite and for tracing the geographical origin. The genetic polymorphisms of *E. canadensis* in Europe will be evaluated by a DNA sequencing analysis. Genes for the RNA polymerase II second largest subunit (rpb2), phosphoenolpyruvate carboxykinase (pepck), and DNA polymerase delta (pold), will be selected as targets for nuclear markers in *E. canadensis*, because of their single-copy nature in many eukaryotic organisms. The main purpose of this study will be to characterize the population genetic structure of this species by microvariant analysis. Data derived from this analysis will be useful for developing new

molecular-based tools, with improved specificity and sensitivity, in the diagnosis of this parasite.

Objectives:	*Knowledge on spread and host range of species inducing cystic echinococcosis in EU*
Expected outputs:	*Acquisition of epidemiological indicators*
Performance indicators:	*Number of characterized parasites*

2.9 Development of analytical methods for the identification of taenidae eggs in the definitive host (dog) faeces

The cestode family Taeniidae consists of two zoonotic genera, *Taenia* and *Echinococcus*. The genus *Echinococcus* is monophyletic due to a remarkable similarity in morphology, features of development, and genetic makeup. By contrast, *Taenia* is a highly diverse group containing approximately 42 valid species and 3 subspecies. In contrast to many other helminth infections, an intra vita diagnosis of taeniidae tapeworm infections cannot reliably be achieved by the microscopical detection of the worm eggs in faecal samples by routine coprological methods (e.g., flotation technique) because eggs of all species of the family Taeniidae are morphologically indistinguishable from one another. Developing a molecular method for detecting and distinguishing between taeniid eggs present in faeces is considered to be essential. After the flotation technique which was already established in the lab, DNA will be extracted from 0.5 to 1.0 g of pellet derived from the flotation of the faecal sample containing taenidae eggs and concentrated in 50 µl volume by conventional DNA extraction kits. Three markers belonging to two genes (nad1 and rrnS genes) will be amplified by a multiplex PCR. 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. This protocol was successfully applied to faecal samples spiked with *E. multilocularis* and *Taenia* spp. Now, it will be applied to faecal samples spiked with *E. granulosus*. Lastly, the flotation-amplification method will be validated on naturally infected dogs.

Objectives:	*Development of analytical methods for the identification of taenidae eggs in the definitive host (dogs) faeces*
Expected outputs:	*Identification of a panel of primers for the molecular identification of eggs*
Performance indicators:	*Number of amplified sequences*

3 Interlaboratory comparison studies

According to the requests of NRLs expressed in the course of the eighth NRL workshop, held in Rome from 23 to 24 May, 2013, four proficiency tests (PTs) will be organised by the EURLP in the course of 2014.

3.1 *Trichinella*

3.1.1 PT on *Trichinella* larva detection in meat samples

The eighth PT on the detection of *Trichinella* larvae in meat samples, will be organised among NRLs to evaluate the competence of NRLs. Test samples (100 or 35 g meatballs made with diaphragm tissue from pigs and/or horses) will be spiked with a known number of *T. spiralis* larvae obtained from experimentally infected mice. Each NRL will receive samples containing two 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.

Objectives:	*To evaluate the PT performance of the NRL personnel*
Expected outputs:	*Increasing sensitivity of the method and skill of the NRL personnel*
Performance indicators:	*Percentage of positive results in comparison to the % detected in the previous years*

3.1.2 PT on *Trichinella* larva identification

The fourth 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 occasionally imported from non-EU countries into Europe, will be collected from infected mice. Vials will be coded and forwarded to participating labs for molecular identification according to the PCR method used in each laboratory. Participant laboratories will be invited to identify single larvae instead of a pool of larvae.

Objectives:	*To evaluate the PT performance of the NRL personnel*
Expected outputs:	*Increasing sensitivity and specificity of the method and skill of the NRL personnel*
Performance indicators:	*% of positive results in comparison to the % detected in the previous years*

3.2 *Echinococcus*

3.2.1 PT on the detection of *Echinococcus* adult worms in intestinal contents

For the fifth 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 three samples. 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.

Objectives:	*To evaluate the PT performance of the NRL personnel*
Expected outputs:	*Increasing sensitivity of the method and skill of the NRL personnel*
Performance indicators:	*% of positive results in comparison to the % detected in the previous years*

3.3 Anisakidae

3.3.1 PT on the detection of Anisakidae larvae in fish fillets

The PT to detect Anisakidae larvae in fish fillets by digestion will be organised for the third time. Anisakidae larvae will be collected from naturally infected fish on the market. A known number of larvae will be spiked in fillets from farmed fish, known to be negative for Anisakidae larvae. 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. Participating NRLs will digest the fish fillets and count the larvae. 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.

Objectives:	*To evaluate the PT performance of the NRL personnel*
Expected outputs:	*Increasing sensitivity of the method and skill of the NRL personnel*
Performance indicators:	*% of positive results in comparison to the % detected in the previous years*

4 Workshop

In the first half of 2014, a two day-workshop will be held at the Istituto Superiore di Sanità of Rome, or in another venue, 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 acquisitions on the epidemiology, diagnosis and control of these pathogens.

Objectives:	*To exchange the epidemiological and diagnostic information on foodborne parasites circulating in EU or at risk to be imported in the EU; training of NRL personnel on foodborne parasites*
Expected outputs:	*NRL staff training*
Performance indicators:	*Appreciation of the workshop by the NRL staff*

5 Visit to NRLs

Qualified personnel of the EURLP will visit 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.

Objectives:	*Exchange information between NRL and EURLP, collection of epidemiological information on foodborne parasites circulating in the MS, identification of strengths and weaknesses of the NRL*
Expected outputs:	*Improvement of the lab weaknesses, acquisition of epidemiological information*
Performance indicators:	*Increasing number of diagnostic tests and increasing contact within the NRL-EURLP network*

6 Training for the personnel of NRLs and developing countries

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

Objectives:	*Training of personnel in the field of foodborne parasites*
Expected outputs:	*Increasing specificity and sensibility of diagnostic tests to detect foodborne parasites*
Performance indicators:	*Increasing reporting data on the epidemiology of foodborne parasites*

7 Update of the website of the EURL for parasites

The website will be updated by publishing the newly developed methods and SOPs to be accredited in 2014, and all presentations displayed during the next Ninth Annual Workshop to be held in Rome on May, 2014. Moreover, educational sheets

on the life cycle, epidemiology, diagnosis and distribution of foodborne parasites will be published in the section "*Foodborne parasites*".

Objectives:	*Continuous improvement of the EURLP web site*
Expected outputs:	*Increase of the available information and its friendly use*
Performance indicators:	*Number of the EURLP web site visitors*

8 Standardization of methods for the detection of parasites in food

A EURLP representative will participate to the next ISO/TC 34/SC 9 meeting to be held in Washington, USA, at the end of June, 2014, in order to report as project leader of the ISO/TC34/SC9/WG6 subgroup *Trichinella* the ongoing standardization process on *Trichinella*. The draft International Standard (DIS): Microbiology of food and animal feed — Detection of *Trichinella* Larvae in meat — Physical method by digestion", sent for voting and comment to ISO and CEN on the basis of Vienna agreement, will hopefully be published at the beginning of 2014. Concerning the standardization process of *Trichinella* serology, on the basis of the results of the inter-laboratory study among 5 expert laboratories (USA, Canada, Italy, Germany and France) aimed at the standardization of antigens and sera, the expert group will proceed with the drafting of the international standard on ELISA method for the detection of *Trichinella* antibodies in swine sera.

Objectives:	*To standardize methods for the detection of parasites in food*
Expected outputs:	*Standardization of the digestion method for the detection of* Trichinella *larvae in meat and for the detection of anti-*Trichinella IgG in swine sera
Performance indicators:	*Publication of the 'Digestion method for the detection of* Trichinella *larvae in meat' as an ISO standard*

9 Validation of commercial apparatuses and kits for the diagnosis of *Trichinella* infections

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 NRL 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 also validated according to the company requests.

Objectives:	*Validation of apparatuses and kits for the diagnoses of foodborne parasites*
Expected outputs:	*Validation of new commercial apparatuses and/or kits*
Performance indicators:	*Publication of the technical report of the validation process*

10 Quality assurance system

The continuous improvement of the EURLP Quality Assurance System is a key point to assure to the NRLs the highest level of reliability of the EURLP services. For this aim, the EURLP in February 2013 applied for the accreditation according to the ISO 17043:2010 standard as Proficiency Testing Provider. The accreditation procedure by the Italian accreditation body, ACCREDIA, is going on, and the EURLP will organize the next proficiency testing rounds according to its accredited quality assurance system, in conformity with the relevant international standard.

Objectives:	*Improvement and control of the EURLP activities and management*
Expected outputs:	*Validation and accreditation of new diagnostic methods in the field of foodborne parasites;*
Performance indicators:	*Accreditation according to ISO 17043:2010 standard*

11 Support to International Institutions

Qualified personnel of the EURLP will support the ECDC, EFSA, FAO, OIE, WHO, and other international institutions in the field of foodborne parasitic zoonoses.

Objectives:	*Scientific and technical support to international Institutions in the field of foodborne parasites*
Expected outputs:	*Participations of the EURLP personnel to meetings and working groups organized by international institutions*
Performance indicators:	*Publications of reports*

12 Meeting at the DG SANCO

The Director of EURL for Parasites or a person designed by the director, will attend the yearly meeting at the DG SANCO.

Rome, 7th August, 2013

European Union Reference Laboratory for Parasites
Istituto Superiore di Sanità
Rome, Italy

The Director of EURL for Parasites
Dr. Edoardo Pozio