Report on the second NRL Proficiency Test to detect adult worms of *Echinococcus* sp. in the intestinal mucosa of the definitive host

March-April, 2010

Table of contents

1 Introduction

Cestode worms of the genus *Echinococcus* are zoonotic parasites circulating in most of the European countries in both wild and domestic animals (Eckert et al., 2001). Humans acquire the infection by the ingestion of eggs shed by dogs, which can contaminate raw or undercooked vegetables and fruits, fomities, and the dog coat. Herbivore and omnivore animals (e.g. sheep, goats, cattle, pigs) are the intermediate hosts of parasites belonging to the *Echinococcus granulosus* group; whereas, sylvatic rodents are the intermediate hosts of parasites belonging to *Echinococcus multilocularis*. Humans can accidentally acquire the infection as an intermediate host, even if he represent a dead end of the parasite cycle. Domestic dogs and sylvatic canides (e.g. red foxes and raccoon dogs) are the final hosts of *E. multilocularis*. Domestic and stray dogs and, rarely wolves, are the final hosts of *E. granulosus*. The incidence of infection greatly varies from one to another MS. In endemic EU countries the incidence can reach 6.3 cases for 100,000 inhabitants (Pozio, 2008).

2 Scope

One of the core duties of the CRLP is to organise proficiency test, as it is stated in the Regulation (EC) No 882/2004 of the European Parliament and of the Council (Commission Regulation No. 882/2004). The scope of this comparison is to test the competence of the appointed NRLs to identify adult worms or their parts, e.g. proglottids and rostrellum, of *Echinococcus* sp. in intestinal mucosa collected from the gut of the final host and to differentiate this worm from other material of parasitic and non-parasitic origin present in the matrice.

3 Time frame

The proficiency test was announced to NRLs by email on February 12, 2010 and the dead line to send the participation form was February 28, 2010. On March 15, 2010, the samples were dispatched to participants by an international courier. Reporting deadline was April 7, 2010.

4 Test material

Foxes shot by hunters in Brandenburg, Germany, in 2009 were collected, stored frozen at -80°C for at least one week (to kill the embryo) and then at -20° until one day before necropsy. The mucosa of the small intestine of 25 foxes found infected with a high worm burden (>50 adult of *E. multilocularis*) was collected (Annex 1). Ten samples were homogenised to prepare a highly positive sample. The same was done with the mucosa with an intermediate worm burden (10-30 adult parasites) to prepare a weakly positive sample. Negative mucosa was used to prepare negative sample. The test material forwarded to each laboratory, consisted of 3 vials containing: 1. *Echinococcus* negative mucosa, this sample was considered to be the negative

control; 2. a weakly *Echinococcus* positive mucosa; and 3. a highly *Echinococcus* positive mucosa (Annex 1). This material was kindly provided by Dr. F. Conraths of the Friedrich-Loeffler Institut, Bundesforschungs Institut fur Tiergesun, Bundesinstitut Fur Risikobewertung (Germany) (Annex 1). In addition, a vial containing cleaned adult worms of *E. multilocularis* collected from the gut of hunted foxes from Switzerland, was provided as positive control (Annex 1). This material was kindly provided by Prof. Peter Deplazes of the Institute of Parasitology, University of Zurich, Switzerland. The positive control and the adult worms used to spike the two samples were preserved in 70% ethanol. The four vials were forwarded in the same package where the samples of the proficiency test for *Trichinella* were sent. All the samples were delivered within 24-36 hours. In the package, the following forms and a letter were included:

1) letter with information on the Proficiency Test and its purpose (Annex 2);
2) package content and its condition of preservation (form 1, Annex 2)
3) laboratory description (form 2, Annex 2);
4) instructions for the detection of *Echinococcus* sp. (form 3, Annex 2);
5) results (form 4, Annex 2);

5 Instructions to participants

Practical instructions were given to all the participants in the form 3 that accompanied the samples and in the letter. To make comparable the results obtained by the different laboratories involved in the proficiency test, all the participants should follow the protocol step by step or, on the contrary, describe the variation.

6 Participating laboratories

Of the 27 MS, Luxembourg appointed the Belgium NRL for parasites, Germany, Malta, and Romania declared not to be interested in this proficiency test, consequently 23 laboratories agreed to participate.

Table 1 – National Reference Laboratories (NRL) participating at the proficiency test for *Echinococcus* sp.

National Reference Laboratories	Country
Institut für Veterinärmedizin, Innsbruck	Austria
Institute of Tropical Medicine, Antwerp	Belgium
National Diagnostic and Research Veterinary Institute, Sofia	Bulgaria
State Veterinary Laboratory, Nicosia	Cyprus
University of Veterinary and Pharm Sciences, Brno	Czech Rep
Danish Food and Veterinary Institute, Copenhagen	Denmark
Estonian Veterinary and Food Laboratory, Tartu	Estonia
Finnish Food Safety, Evira, Oulu	Finland
Lab. d'études et de recherches sur la rage et pathologie des animaux sauvages,	France

AFSSA, Malzeville	
Centre of Athens Veterinary Institutions, Athens	Greece
Laboratories for Parasitology, Fish and Bee Diseases, Budapest	Hungary
Veterinary Laboratory, Department of Agriculture & Food Laboratories, Celbridge, County Kildare	Ireland
Istituto Zooprofilattico Sperimentale of Sardinia, Sassari	Italy
Institute of Food Safety, Animal Health and Environment, BIOR	Latvia
National Veterinary Laboratory, Vilnius	Lithuania
National Institute of Public Health and the Environment, RIVM, Bilthoven	Netherlands
National Veterinary Research Institute, Pulawy	Poland
Laboratório Nacional de Investigação Veterinária, Lisboa	Portugal
State Veterinary and Food Institute, Bratislava	Slovak Rep.
National Veterinary Institute, Ljubljana	Slovenia
Centro Nacional de Alimentación. Agencia Española de Seguridad Alimentaria y Nutrición, Majadahonda	Spain
Statens Veterinärmedicinska Anstalt, Uppsala	Sweden
Veterinary Laboratories Agency, Weybridge	UK

7 Results

Of the 23 NRLs which agreed to participate to the proficiency test, 14 (61%) are accredited according to ISO/IEC 17025:2005. Of them, 8 were accredited in the last 5 years and 6 from more than 5 years. Three laboratories of 23 (13%) have accredited a diagnostic test to detect *Echinococcus* larvae/adults in the intermediate/definitive hosts. Seventy persons are currently working with Echinococcus: 37 scientist and 33 technicians. The methods used at the NRLs to detect adult worms in the intermediate and definitive hosts are shown in the Annex 3.

Twenty-two out of twenty-three NRLs (96%) identified correctly all the three samples (Annex 4). Sixteen laboratories (70%) correctly specify that sample 1 was highly positive and sample 2 was low positive.

8 Conclusions

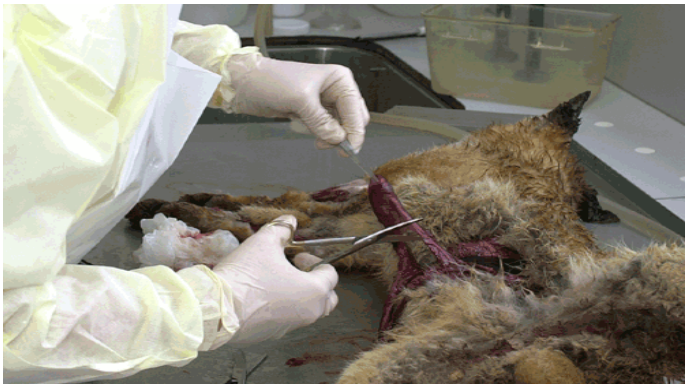
The experience derived from the first proficiency test suggested to fix adult worms in ethanol 70% for the identification of samples. In fact, this second proficiency test demonstrated that the personnel of NRLs has good ability to detect this parasite in a qualitatively test. In the future a quantitative test should be useful to better measure the skillness of the NRLs.

9 References

Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, Z.S. (2001). WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organisation for Animal Health, Paris, France, pp. 1- 265.

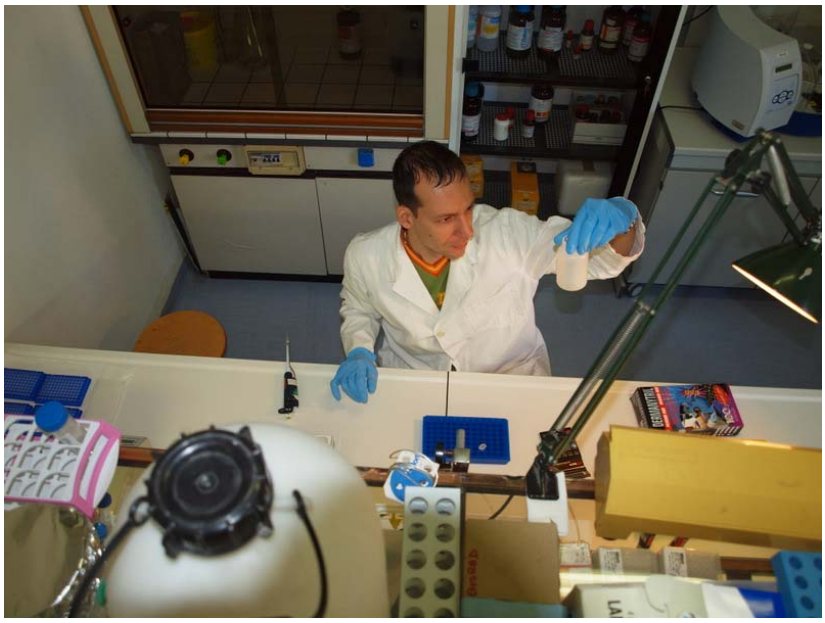
Pozio, E. (2008). Epidemiology and control prospects of foodborne parasitic zoonoses in the European Union. Parassitologia 50:17-24.

Annex 1



Collection of mucosa from foxes in order to prepare positive samples for the proficiency test

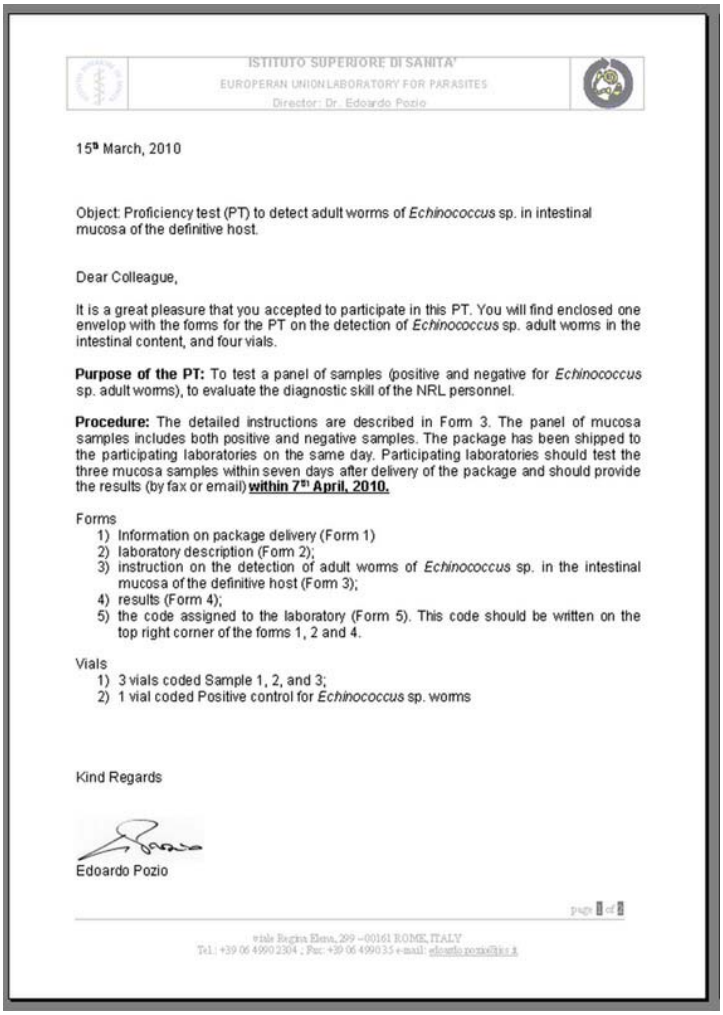
Adult *Echinococcus* worms isolated from the mucosa of foxes



Mucosa homogenised to prepare highly and weakly positive samples.

Four vials containing: 1. adult *Echinococcus* worms as positive control; 2. *Echinococcus* negative mucosa; 3. a weakly *Echinococcus* positive mucosa; and 4. a highly *Echinococcus* positive mucosa.

Annex 2



ISTITUTO SUPERIORE DI SANITA'
EUROPEAN UNION LABORATORY FOR PARASITES
Director: Dr. Edoardo Pozio

15th March, 2010

Object: Proficiency test (PT) to detect adult worms of *Echinococcus* sp. in intestinal mucosa of the definitive host.

Dear Colleague,

It is a great pleasure that you accepted to participate in this PT. You will find enclosed one envelop with the forms for the PT on the detection of *Echinococcus* sp. adult worms in the intestinal content, and four vials.

Purpose of the PT: To test a panel of samples (positive and negative for *Echinococcus* sp. adult worms), to evaluate the diagnostic skill of the NRL personnel.

Procedure: The detailed instructions are described in Form 3. The panel of mucosa samples includes both positive and negative samples. The package has been shipped to the participating laboratories on the same day. Participating laboratories should test the three mucosa samples within seven days after delivery of the package and should provide the results (by fax or email) **within 7th April, 2010.**

Forms
1) Information on package delivery (Form 1)
2) laboratory description (Form 2);
3) instruction on the detection of adult worms of *Echinococcus* sp. in the intestinal mucosa of the definitive host (Form 3);
4) results (Form 4);
5) the code assigned to the laboratory (Form 5). This code should be written on the top right corner of the forms 1, 2 and 4.

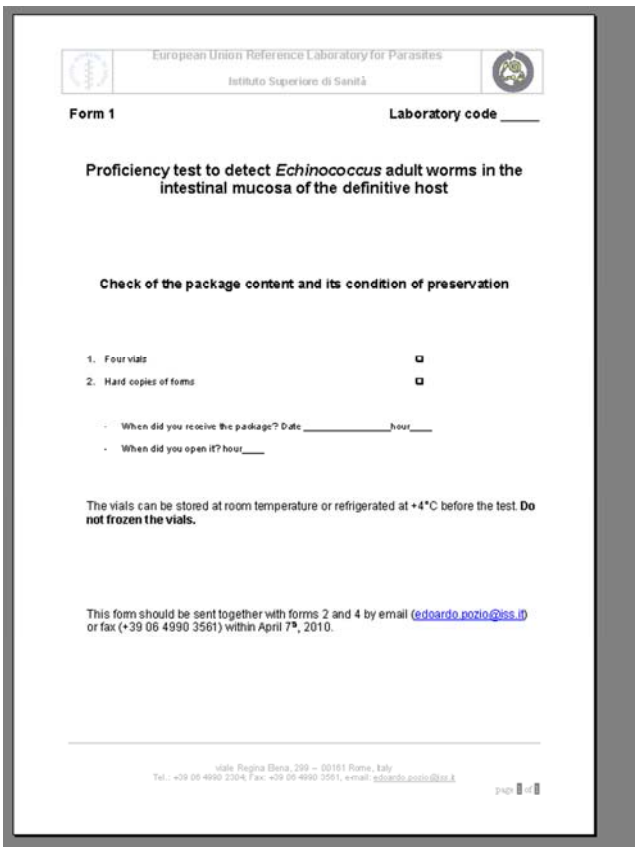
Vials
1) 3 vials coded Sample 1, 2, and 3;
2) 1 vial coded Positive control for *Echinococcus* sp. worms

Kind Regards

Edoardo Pozio

page 1 of 2

viale Regina Elena, 299 – 00161 ROME, ITALY
Tel.: +39 06 4990 2304 ; Fax: +39 06 4990 35 e-mail: edoardo.pozio@iss.it



European Union Reference Laboratory for Parasites

Istituto Superiore di Sanità

Form 1 **Laboratory code _____**

Proficiency test to detect *Echinococcus* adult worms in the intestinal mucosa of the definitive host

Check of the package content and its condition of preservation

1. Four vials ☐
2. Hard copies of forms ☐

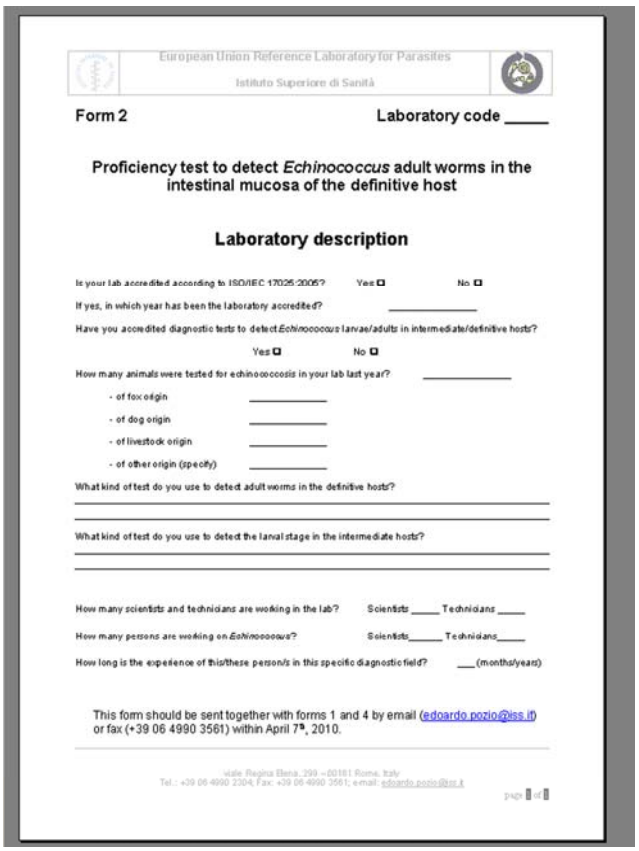
- When did you receive the package? Date _____hour____
- When did you open it? hour____

The vials can be stored at room temperature or refrigerated at +4°C before the test. **Do not frozen the vials.**

This form should be sent together with forms 2 and 4 by email (edoardo.pozio@iss.it) or fax (+39 06 4990 3561) within April 7th, 2010.

viale Regina Elena, 299 – 00161 Rome, Italy
Tel.: +39 06 4990 2304; Fax: +39 06 4990 3561; e-mail: edoardo.pozio@iss.it

page ▮ of ▮



European Union Reference Laboratory for Parasites

Istituto Superiore di Sanità

Form 2 **Laboratory code _____**

Proficiency test to detect *Echinococcus* adult worms in the intestinal mucosa of the definitive host

Laboratory description

Is your lab accredited according to ISO/IEC 17025:2005? Yes ☐ No ☐

If yes, in which year has been the laboratory accredited? _____

Have you accredited diagnostic tests to detect *Echinococcus* larvae/adults in intermediate/definitive hosts?

Yes ☐ No ☐

How many animals were tested for echinococcosis in your lab last year? _____

- of fox origin _____
- of dog origin _____
- of livestock origin _____
- of other origin (specify) _____

What kind of test do you use to detect adult worms in the definitive hosts?

_____

_____

What kind of test do you use to detect the larval stage in the intermediate hosts?

_____

_____

How many scientists and technicians are working in the lab? Scientists _____ Technicians _____

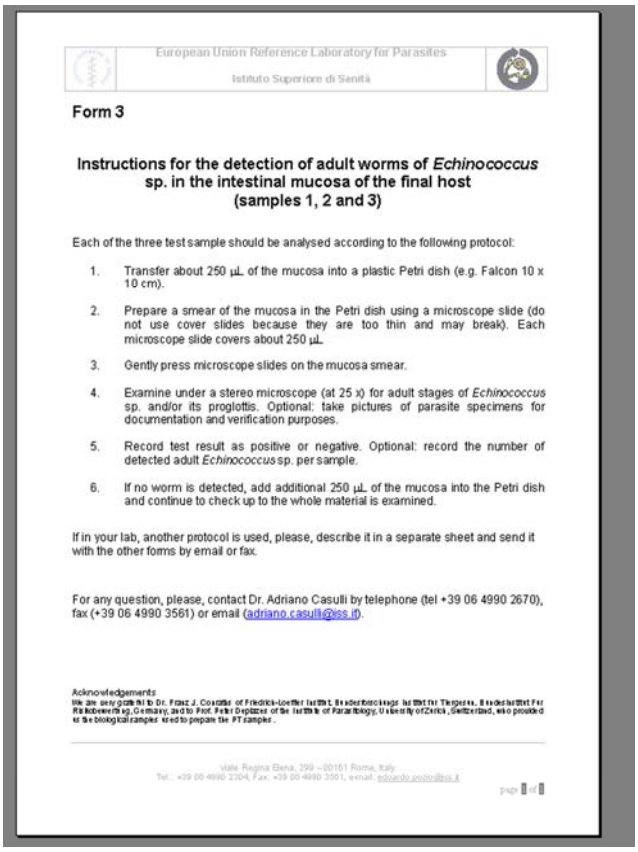
How many persons are working on *Echinococcus*? Scientists_____ Technicians_____

How long is the experience of this/these person/s in this specific diagnostic field? ___(months/years)

This form should be sent together with forms 1 and 4 by email (edoardo.pozio@iss.it) or fax (+39 06 4990 3561) within April 7th, 2010.

viale Regina Elena, 299 – 00161 Rome, Italy
Tel.: +39 06 4990 2304; Fax: +39 06 4990 3561; e-mail: edoardo.pozio@iss.it

page ▮ of ▮



European Union Reference Laboratory for Parasites

Istituto Superiore di Sanità

Form 3

Instructions for the detection of adult worms of *Echinococcus* sp. in the intestinal mucosa of the final host (samples 1, 2 and 3)

Each of the three test sample should be analysed according to the following protocol:

1. Transfer about 250 µL of the mucosa into a plastic Petri dish (e.g. Falcon 10 x 10 cm).
2. Prepare a smear of the mucosa in the Petri dish using a microscope slide (do not use cover slides because they are too thin and may break). Each microscope slide covers about 250 µL.
3. Gently press microscope slides on the mucosa smear.
4. Examine under a stereo microscope (at 25 x) for adult stages of *Echinococcus* sp. and/or its proglottis. Optional: take pictures of parasite specimens for documentation and verification purposes.
5. Record test result as positive or negative. Optional: record the number of detected adult *Echinococcus* sp. per sample.
6. If no worm is detected, add additional 250 µL of the mucosa into the Petri dish and continue to check up to the whole material is examined.

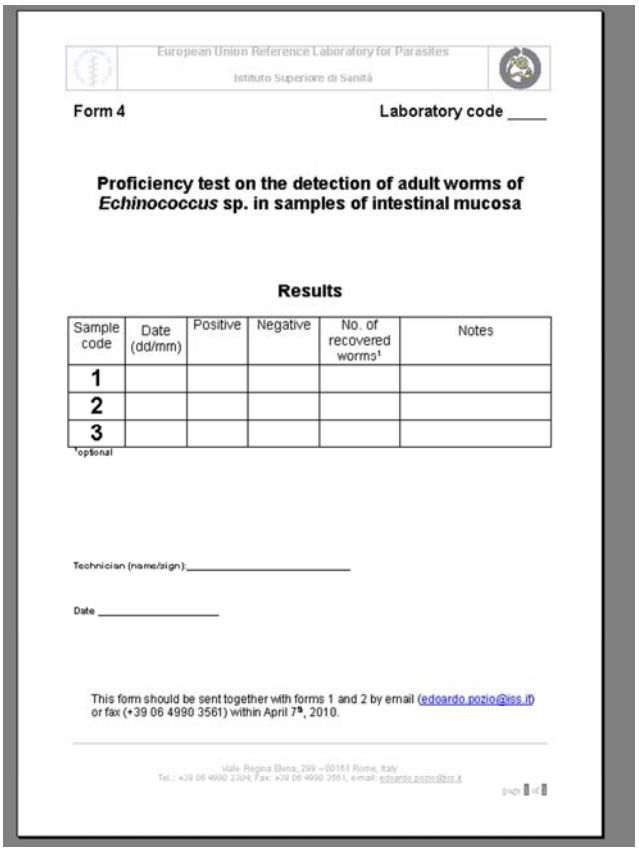
If in your lab, another protocol is used, please, describe it in a separate sheet and send it with the other forms by email or fax.

For any question, please, contact Dr. Adriano Casulli by telephone (tel +39 06 4990 2670), fax (+39 06 4990 3561) or email (adriano.casulli@iss.it).

Acknowledgements
We are very grateful to Dr. Franz J. Conraths of Friedrich-Loeffler Institut, Bundesforschungs Institut für Tiergesund, Bundesinstitut für Risikobewertung, Germany, and to Prof. Peter Deplazes of the Institute of Parasitology, University of Zurich, Switzerland, who provided us the biological samples used to prepare the PT samples.

viale Regina Elena, 299 – 00161 Rome, Italy
Tel.: +39 06 4990 2304; Fax: +39 06 4990 3561; e-mail: edoardo.pozio@iss.it

page ▮ of ▮



European Union Reference Laboratory for Parasites

Istituto Superiore di Sanità

Form 4 **Laboratory code _____**

Proficiency test on the detection of adult worms of *Echinococcus* sp. in samples of intestinal mucosa

Results

Sample code	Date (dd/mm)	Positive	Negative	No. of recovered worms[1]	Notes
1					
2					
3					

[1]optional

Technician (name/sign):_____

Date _____

This form should be sent together with forms 1 and 2 by email (edoardo.pozio@iss.it) or fax (+39 06 4990 3561) within April 7th, 2010.

viale Regina Elena, 299 – 00161 Rome, Italy
Tel.: +39 06 4990 2304; Fax: +39 06 4990 3561; e-mail: edoardo.pozio@iss.it

page ▮ of ▮

Annex 3

Methods used routinely by the participating NRLs to detect *Echinococcus* sp. in the intermediate hosts

Laboratory	Method to detect *Echinococcus* sp. in intermediate hosts
1E	MICROSCOPY
2E	NECROSCOPY
3E	NECROSCOPY
5E	MICROSCOPY
6E	MICROSCOPY
7E	No method routinely performed
9E	Not reported
11E	NECROSCOPY
12E	No method routinely performed
13E	No method routinely performed
14E	No method routinely performed
16E	No method routinely performed
18E	MICROSCOPY
19E	MICROSCOPY
20E	NECROSCOPY + PCR
22E	No method routinely performed
23E	MICROSCOPY
25E	MICROSCOPY
29E	NECROSCOPY + PCR
30E	No method routinely performed
31E	MICROSCOPY + PCR
32E	MICROSCOPY + PCR
33E	Not reported

Methods used routinely by the participating NRLs to detect *Echinococcus* sp. in the definitive host

Laboratory	Method to detect *Echinococcus* sp. in definitive hosts
1E	SCT[1]
2E	NECROPSY
3E	MICROSCOPY
5E	IST[2]
6E	PCR
7E	SCT
9E	Not reported
11E	Dog treatment with Arecoline
12E	No method routinely performed
13E	PCR
14E	No method routinely performed
16E	No method routinely performed
18E	MICROSCOPY
19E	SCT
20E	SCT + PCR
22E	No method routinely performed
23E	No method routinely performed
25E	FLOTATION
29E	IST + SCT
30E	SCT
31E	SCT
32E	SCT + IST + PCR
33E	Not reported

[1]SCT = sedimentation and counting technique

[2]IST = intestinal scraping technique

Annex 4

Proficiency Test Results

Laboratory	SAMPLE 1 Highly Positive	SAMPLE 2 Low Positive	SAMPLE 3 Negative
1E	Highly Positive	Low Positive	Negative
2E	Positive	Positive	Negative
3E	Highly Positive	Low Positive	Negative
5E	Highly Positive	Low Positive	Negative
6E	Highly Positive	Low Positive	Negative
7E	Positive	**Negative**	Negative
9E	Positive	Positive	Negative
11E	Positive	Positive	Negative
12E	Highly Positive	Low Positive	Negative
13E	Highly Positive	Low Positive	Negative
14E	Positive	Positive	Negative
16E	Highly Positive	Low Positive	Negative
18E	Highly Positive	Low Positive	Negative
19E	Highly Positive	Low Positive	Negative
20E	Highly Positive	Low Positive	Negative
22E	Positive	Positive	Negative
23E	Highly Positive	Low Positive	Negative
25E	Positive	Positive	Negative
29E	Highly Positive	Low Positive	Negative
30E	Highly Positive	Low Positive	Negative
31E	Highly Positive	Low Positive	Negative
32E	Highly Positive	Low Positive	Negative
33E	Highly Positive	Low Positive	Negative