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

## March-April, 2011

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## 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 they represent a dead end of the parasite cycle. Domestic dogs and sylvatic canids (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 EURLP is to organise proficiency tests (PTs), 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 PT 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 (PT) was announced to NRLs by email on 17 January, 2011 and the dead line to send the participation form was 12 February, 2011. On March 21, 2011, the samples were dispatched to participants by an international courier. Reporting deadline was 4$^{th}$ April, 2011.

## 4 Test material

From November 2010 to February 2011, carcasses of red foxes shot by hunters in Lublin province, Poland, were sent to the Department of Parasitology and Invasive Diseases National Veterinary Research Institute in Pulawy, Poland. Carcasses were forwarded in individual plastic bags at +4 °C. The intestinal tract was removed and stored at −20 °C. Then for safety reasons (i.e. to kill the *Echinococcus* embryos eventually present in the gut), the intestinal tract was frozen at −80 °C for 7 days before examination. After freezing, the gut was thawed at room temperature and the middle and posterior parts of the intestine were collected and tested by sedimentation and counting technique (SCT) according to a previous published

protocol (Mathis et al., 1996). If the sample resulted negative, the anterior third part of the intestine was opened and the mucosa was scraped and autoclaved for the reduction of bacterial activity. This material was kindly provided to the EURLP by Dr. Jacek Karamon of the National Veterinary Research Institute, Pulawy, Poland. The mucosa of the small intestine of 22 foxes found to be negative was infected with 10 or 30 worms in order to prepare weakly or highly positive samples, respectively (Annex 1). 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 being considered the negative control; 2. a weakly (n= 10) *Echinococcus* positive mucosa; and 3. a highly (N=30) *Echinococcus* positive mucosa (Annex 1). All samples were delivered within 24-36 hours. In the package, a letter and the following forms were included:

1) letter with information on PT 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);
6) laboratory code.

## 5 Instructions to participants

Practical instructions were given to all the participants in the form 3 and in the accompanying letter. To make the results obtained by laboratories comparable, all participants had to follow the protocol step by step or describe all modification made, if any. It was requested to quantitatively evaluate the samples by one of the two suggested methods: 1) Intestinal Scraping Technique (IST); and 2) Sedimentation and Counting Technique (SCT); (Mathis et al., 1996; Eckert et al., 2001).

## 6 Participating laboratories

Eighteen laboratories agreed to participate (see Annex 3).

## 7 Evaluation criteria

- for samples with 30 adult worms, the count of at least 50% of worms was considered as positive
- for samples with 10 adult worms, the count of at least 40% of worms was considered as positive

## 8 Results

Out of the 18 NRLs which agreed to participate to the proficiency test, 16 (89%) are accredited according to ISO/IEC 17025:2005 and 4 (22%) have accredited a diagnostic test to detect *Echinococcus* larvae/adults in the intermediate/definitive hosts. Seventy-four persons are currently working with *Echinococcus*: 36 scientist and 28 technicians.

Out of the 18 participating NRLs, 11 (61%) labs tested the samples by the SCT and 7 (39%) labs by IST (Annex 4). One lab (code E22) tested the three samples only qualitatively. The average recovery rate of adult worms from the weakly spiked sample was 6 (% of recovery 60; range 1-10), whereas in the highly spiked sample the average recovery rate was 21 (% of recovery 70; range 2-29).

Results obtained by the NRLs (Annex 4):

- Sample 1 (negative sample): 18 laboratories (100%) obtained correct results.
- Sample 2 (spiked with 10 worms): 13 laboratories (76.5%) obtained correct results.
- Sample 3 (spiked with 30 worms): 14 laboratories (82.3%) obtained correct results.

## 9 Conclusions

The experience derived from the second PT on  carried out in 2010, showed that the personnel of NRLs had good ability to detect this parasite in a qualitative test. For this reason for the third PT, it was established to request a quantitative test spiking the samples with a known number of adult worms of *Echinococcus*. Thirteen (72.2%) labs detected at least 40% of the worms present in low infected sample and 14 (77.8%) detected at least 50% of the worms present in highly infected sample.

## 10 References

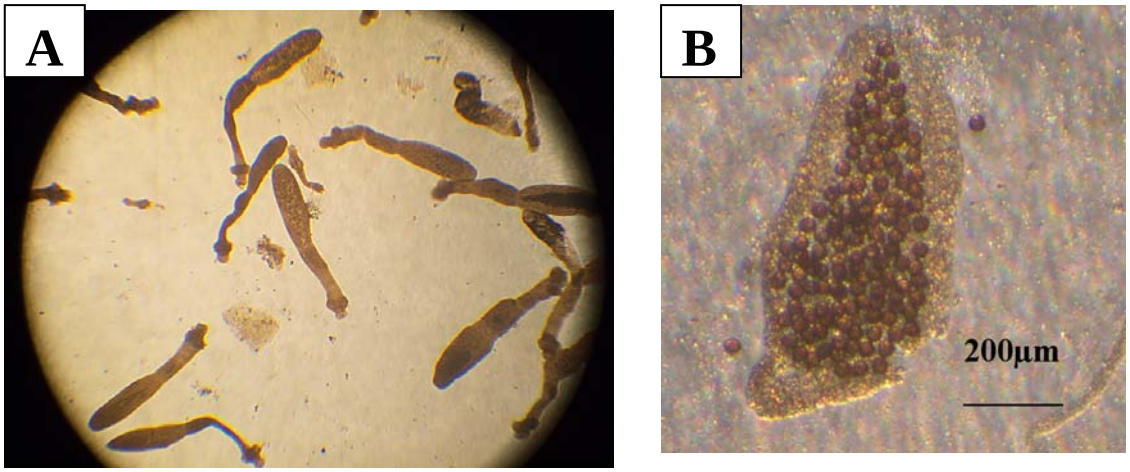
Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, Z.S. (2001). WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organisation for Animal Health, Paris, France, pp. 1- 265.

Mathis A, Deplazes P, Eckert J. (1996). An improved test system for PCR-based specific detection of Echinococcus multilocularis eggs. J Helminthol. 70:219-22.

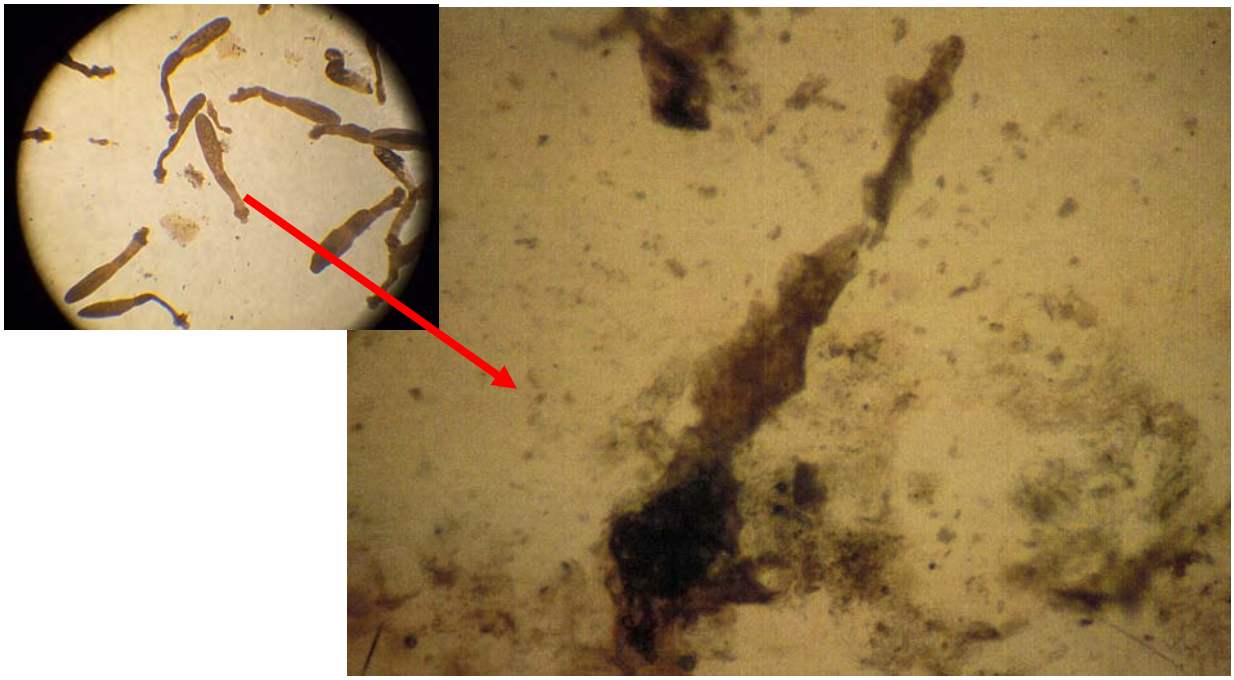
Pozio, E. (2008). Epidemiology and control prospects of foodborne parasitic zoonoses in the European Union. Parassitologia 50:17-24.

# Annex 1

**Necroscopy of a fox carcass to collect the gut in order to prepare the samples for the proficiency test.**



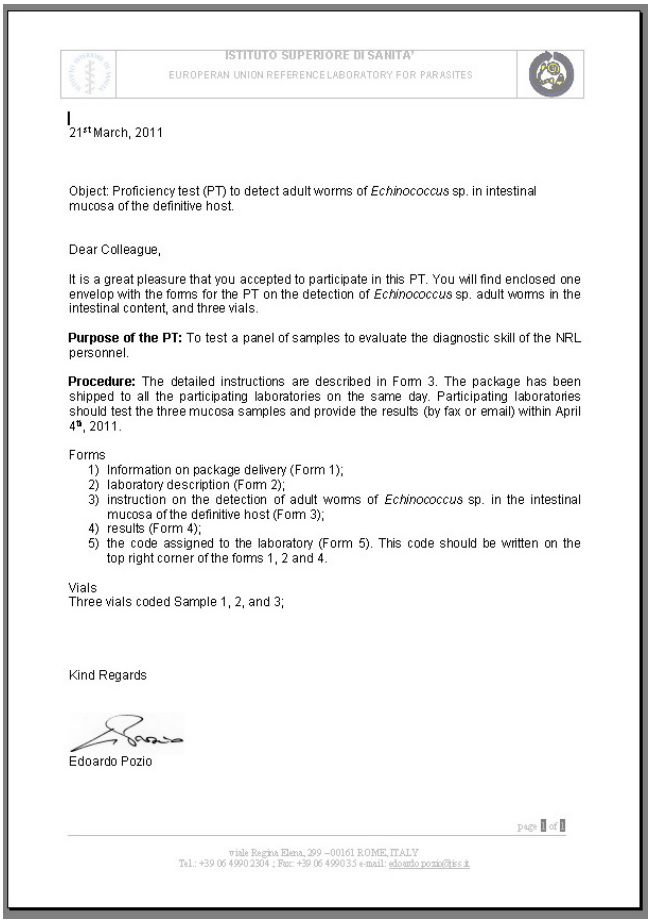

**Adult worms of *Echinococcus* (a) and a proglottid (b) isolated from the intestinal mucosa of a fox**



**Intestinal mucosa spiked with adult worms of *Echinococcus* to prepare highly and weakly positive samples.**

**PT samples: 1. *Echinococcus* negative mucosa; 2. weakly *Echinococcus* positive mucosa spiked with 10 worms; and 3. highly *Echinococcus* positive mucosa spiked with 30 worms.**

# Annex 2



ISTITUTO SUPERIORE DI SANITA'
EUROPEAN UNION REFERENCE LABORATORY FOR PARASITES

21st March, 2011

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 three vials.

**Purpose of the PT:** To test a panel of samples to evaluate the diagnostic skill of the NRL personnel.

**Procedure:** The detailed instructions are described in Form 3. The package has been shipped to all the participating laboratories on the same day. Participating laboratories should test the three mucosa samples and provide the results (by fax or email) within April 4th, 2011.

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
Three vials coded Sample 1, 2, and 3;

Kind Regards

Edoardo Pozio

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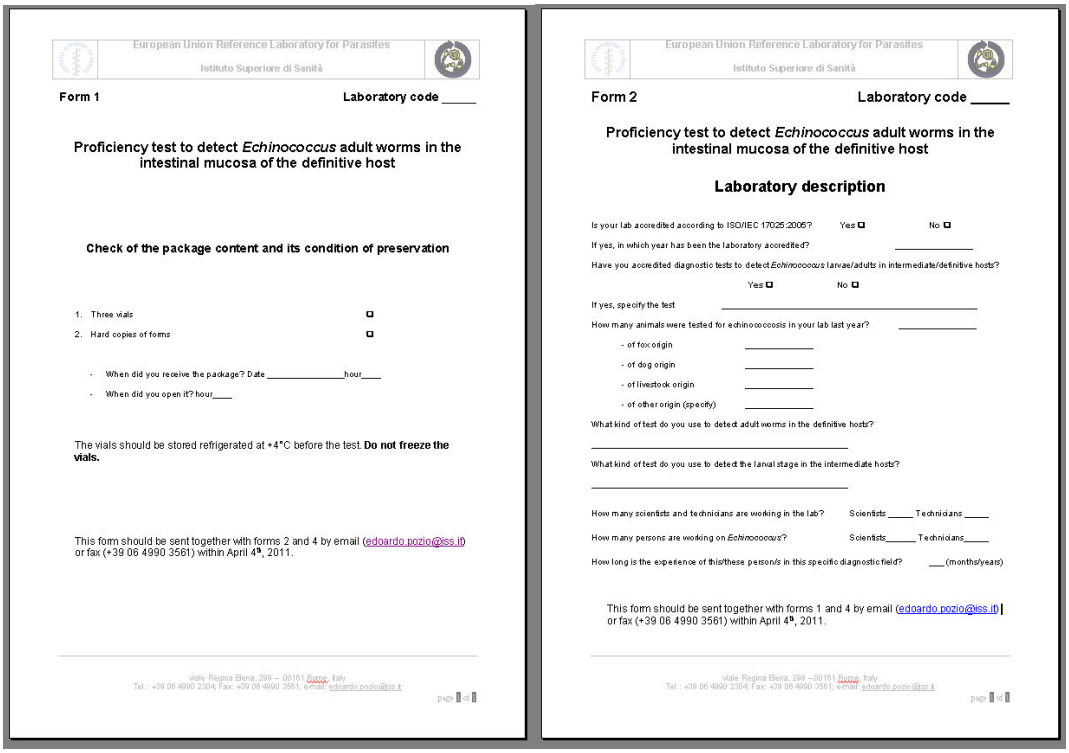

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**Form 1** **Laboratory code _____**

## 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. Three vials ☐
2. Hard copies of forms ☐

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

The vials should be stored refrigerated at +4°C before the test. **Do not freeze 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 4th, 2011.

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**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 ☐

If yes, specify the test _____

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 4th, 2011.

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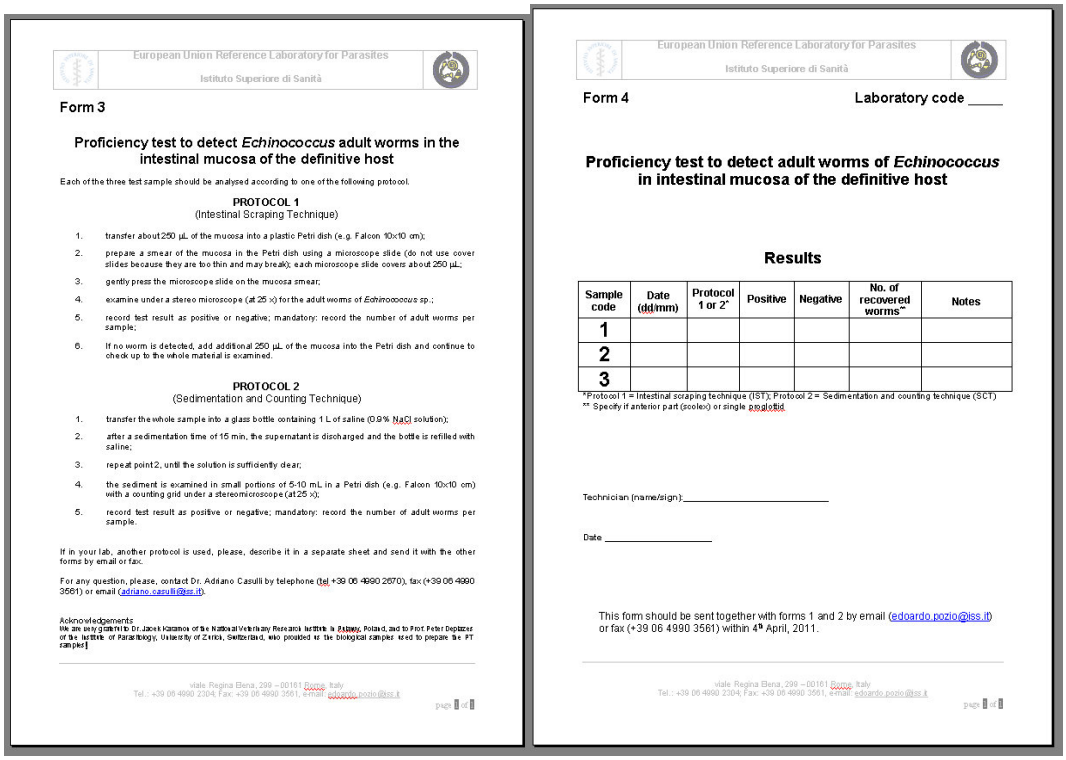

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**Form 3**

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

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

**PROTOCOL 1**
(Intestinal Scraping Technique)

1. transfer about 250 µL of the mucosa into a plastic Petri dish (e.g. Falcon 10x10 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 the microscope slide on the mucosa smear;
4. examine under a stereo microscope (at 25 x) for the adult worms of *Echinococcus* sp.;
5. record test result as positive or negative; mandatory: record the number of adult worms 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.

**PROTOCOL 2**
(Sedimentation and Counting Technique)

1. transfer the whole sample into a glass bottle containing 1 L of saline (0.9% NaCl solution);
2. after a sedimentation time of 15 min, the supernatant is discharged and the bottle is refilled with saline;
3. repeat point 2, until the solution is sufficiently clear;
4. the sediment is examined in small portions of 5-10 mL in a Petri dish (e.g. Falcon 10x10 cm) with a counting grid under a stereomicroscope (at 25 x);
5. record test result as positive or negative; mandatory: record the number of adult worms per sample.

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. Jacek Karamon of the National Veterinary Research Institute in Puławy, Poland, and to Prof. Peter Deplazes of the Institute of Parasitology, University of Zurich, Switzerland, who provided to the biological samples used to prepare the PT samples.

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**Form 4** **Laboratory code _____**

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

### Results

| Sample code | Date (dd/mm) | Protocol 1 or 2* | Positive | Negative | No. of recovered worms** | Notes |
|---|---|---|---|---|---|---|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |

*Protocol 1 = Intestinal scraping technique (IST); Protocol 2 = Sedimentation and counting technique (SCT)
** Specify if anterior part (scolex) or single proglottid

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 4th April, 2011.

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# Annex 3

## 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 |
| Technopole Agricole et Vétérinaire, Malzeville | France |
| Friedrich-Loeffler-Institut, Institut für Epidemiologie | Germany |
| Centre of Athens Veterinary Institutions, Athens | Greece |
| Laboratories for Parasitology, Fish and Bee Diseases, Budapest | Hungary |
| Istituto Zooprofilattico Sperimentale of Sardinia, Sassari | Italy |
| Laboratory of Food and Environmental Investigations, National Diagnostic Centre | Latvia |
| National Veterinary Laboratory, Vilnius | Lithuania |
| National Veterinary Research Institute, Pulawy | Poland |
| National Veterinary Laboratory | Malta |
| Institute for Diagnosis and Animal Health | Romania |

# Annex 4

## Proficiency Test Results

| Laboratory code | Detection method[1] | Number of worms detected | | |
|---|---|---|---|---|
| | | Sample 1 (neg. control) | Sample 2 (10 worms) | Sample 3 (30 worms) |
| E1 | SCT | 0 | 7 | 24 |
| E3 | SCT | 0 | 7 | 2 |
| E5 | SCT | 0 | 5 | 27 |
| E7 | SCT | 0 | 7 | 24 |
| E11 | SCT | 0 | 2 | 25 |
| E12 | IST | 0 | 1 | 22 |
| E13 | SCT | 0 | 9 | 29 |
| E16 | IST | 0 | 2 | 17 |
| E18 | IST | 0 | 9 | 28 |
| E20 | SCT | 0 | 5 | 24 |
| E22 | SCT | 0 | +[2] | ++[2] |
| E23 | IST | 0 | 7 | 14 |
| E24 | SCT | 0 | 9 | 20 |
| E26 | SCT | 0 | 5 | 25 |
| E29 | IST | 0 | 6 | 10 |
| E31 | SCT | 0 | 9 | 23 |
| E37 | IST | 0 | 3 | 17 |
| E39 | IST | 0 | 10 | 27 |

[1] IST = Intestinal Scraping Technique; SCT = Sedimentation and Counting Technique.
[2] The samples were tested only qualitatively, not quantitatively as requested.