PT: "Detection of *Echinococcus spp* worms in the intestinal mucosa of the definitive host"

Procedure

Test samples

Description. Each 12 ml sample consists of 8 ml of homogenized intestinal mucosa plus 4 ml of ethanol 70%, spiked or not with adult worms of *Echinococcus* spp.

Sample preparation.

Matrix. A pool of fox intestines from *E. multilocularis* free-areas is stored at –80°C for 14 days. Intestines are incised longitudinally and examined macroscopically for large helminths, and then cut into 20 cm long segments. The segments are stripped between two pressed fingers and the mucosa is collected in a bottle. The pool of mucosa is homogenized using a mixer. Subsequently, 8 ml of mixed mucosa is pipetted into a 15 ml tube and 4 ml of ethanol 70% is added.

Parasites. In order to avoid the risk of infection for laboratory personnel, the carcasses of foxes or their intestines shall be deep-frozen at – 80°C for at least one week before necropsy. This procedure kills the eggs of *E. multilocularis* if the temperature is retained in all parts of the material for at least 4 days at –70°C, or for 2 days at –80°. Strict safety precautions shall be observed during the whole necropsy procedure. Adult worms belonging to *Echinococcus* spp. are used for the preparation of proficiency panels. Worms are obtained from the small intestine of foxes collected in high endemic areas using the Sedimentation and Counting Technique (SCT) according to WHO-OIE manual on echinococcosis (WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Eckert J et al., eds., 2001):

a) After deep-freezing at – 80°C for 7 days, the small intestine is incised longitudinally and examined macroscopically for large helminths, and then cut into 20 cm long segments;
b) The segments of the intestine are transferred to a glass bottle containing 1 L of physiological saline solution. After vigorous shaking for a few seconds, the mucosa is stripped between two pressed fingers, and the segments of the intestine are removed from the flask.
c) The washing fluid with the intestinal material is sedimented several times for 15 min each, and the supernatant decanted until the sediment is sufficiently cleared from coloured particles.
d) The sediment is examined in small portions of 5-10 ml in rectangular plastic dishes with a counting grid (9 cm × 9 cm Falcon®) under a stereo-microscope at 25X magnification.

Adult worms (up to 4.5 mm long) obtained from SCT, are preserved in ethanol 70% (room temperature) until spiked into PT samples of mucosa .

Worms are counted under a stereo-microscope onto a watch glass (by two different technicians) and transferred to the mucosa samples by rinsing the watch glass with 1 ml ethanol (70%). The watch glass is then observed under the stereo-microscope to verify if some worms are left sticking onto the glass. The 15 ml

tubes are then plugged and sealed with plastic paraffin film, put individually in a plastic bag sealed under vacuum and labeled with an alfanumeric code randomly generated.

Homogeneity check Since proficiency samples for the detection of *Echinococcus spp*. worms by SCT are made by individually spiked samples, homogeneity is ensured by an accurate control of the number of larvae spiked into each sample made by two operators.

Preparation of packages. Each sample is put in a plastic bag sealed under vacuum in order to assure their preservation. Each sample is labeled with an unique code without any indication of the level of contamination or any information on the identity of the testing laboratory. All bags containing the mucosa samples, are put in a larger bag under vacuum, which is put inside a polystyrene carton, ready for shipment. A number of ice packs are placed in the package in order to maintain the inside temperature between 4 and 15°C during transportation.

Stability check and quality control. The stability of the samples in the package has been evaluated by ad hoc experiments made by EURLP. Worms preserved in mucosa containing 70% ethanol, and stored between 4 and 15°C maintain their morphology up to 21 days from the date of preparation.

Criteria for result evaluation

Results evaluation is performed only by qualitative approach: the results of the SCT are reported as “correct” (i.e., detection of one or more *Echinococcus* adult worm in spiked samples) or “incorrect” (false positive or false negative), irrespective of the number of worms in the sample/s. Final evaluation is considered as “positive” if all samples are correctly identified.

Report

Within 10 working days after the due date to submit the results of samples analysis, the EURLP provides an Individual PT Report including the following information: i) the number of spiked worms per sample; ii) the number of worms detected by the laboratory in the sample; iii) a qualitative evaluation iv) final evaluations; and v) recommendations based on the laboratory performance.

The Individual PT Report will be delivered as .pdf file via e-mail or fax.

EURLP also provides the Final PT Report, including results obtained by all participants. The final report is presented to the NRL during the annual workshop and subsequently published on the EURLP website.

To guarantee confidentiality, in the final report laboratories are identified by alphanumeric codes.

For any information or problem related to the PT participation, please address to:

Dr. Adriano Casulli; e-mail: adriano.casulli@iss.it