EFFICACY OF INTESTINAL SCRAPING TECHNIQUE IN THE DETECTION OF *ECHINOCOCCUS MULTILOCULARIS* - ESTIMATION OF THE LIMIT OF THE DETECTION AND COMPARISON WITH SEDIMENTATION AND COUNTING TECHNIQUE

JACEK KARAMON, JACEK SROKA, TOMASZ CENCEK, MACIEJ KOCHANOWSKI, AND JOANNA DĄBROWSKA

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland.

Received: August 1, 2012 Accepted: November 5, 2012

Abstract

The aim of the study was to estimate the effectiveness of intestinal scraping technique (IST) in the detection of *Echinococcus multilocularis*. The experimental assessment of the limit of detection and comparison with “gold standard” (sedimentation and counting technique - SCT) was also performed. Samples of fox small intestines experimentally enriched with known numbers of *E. multilocularis* tapeworms, were used. Twenty four samples containing 10, 30, 60, and 90 *E. multilocularis* tapeworms were prepared. Moreover, in order to compare IST with SCT, 127 intestines of foxes were examined using both methods. The limit of detection was estimated at 30 *E. multilocularis* tapeworms per sample of the intestine. Moreover, mean number of *Echinococcus* found by IST were several dozen times lower than the real content of these tapeworms in the samples (on average only 2 to 3.2% of worms were recovered). Among 127 intestinal samples examined with the use of two methods, eight samples (8.2%) were positive by SCT and only two (1.6%) when IST was used. A relatively high limit of detection estimated experimentally in the first part of the study, as well as, the results obtained in field investigations showed clearly that IST method could significantly decrease the reliability of the results of investigations, especially carried out in regions where a very low prevalence of *E. multilocularis* occurs or in countries, which want to demonstrate that they are free from this parasite.

Key words: *Echinococcus multilocularis*, intestinal scraping technique, efficacy, limits of detection.

*Echinococcus multilocularis* is one of the most dangerous zoonotic factors, which is a significant public health problem. Humans can be accidentally intermediate host in the life cycle of this tapeworm. The larvae of *E. multilocularis*, generally developing in the liver, very often bring to death of the infected person. Invasive eggs, produced by adult tapeworms occurring in the intestines of a definitive host – carnivores, especially foxes, are the source of infection for people. In many countries investigations on the prevalence of these parasites were carried out in foxes to assess the infection risk for people and also to confirm the lack of this parasite in the animals. Different kinds of techniques are available for the detection of these parasites in definitive hosts. There are microscopic post mortem methods, PCR, and ELISA modifications (3; 4, 5; 11, 12, 17, 18). During wide monitoring projects, the post mortem examinations of the small intestines of foxes still appear to be the most popular methods, mainly with regard to their relatively low costs and high effectiveness. Among them, the most sensitive is sedimentation and counting technique (SCT), regarded as the “gold standard”, described by Rausch *et al.* (14) and Hofer *et al.* (7), and now published by the World Organisation for Animal Health in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (13). From these types of methods, OIE recommended also intestinal scraping technique (IST) (4, 6, 13), which is also often used in large prevalence investigations, especially because of it’s not time consuming character (1, 2, 15). However, the short time of examination by this technique is connected with its lower efficacy (6, 7). The real limit of detection by this method has not been estimated yet. It can be assessed only with the use of samples with known numbers of the parasites. Since the intestines from naturally infected animals do not fulfill this requirement, only the method with experimental enrichment of the intestines with tapeworms remains useful for such analysis. Our earlier experience with such kind of estimation showed unexpected limitations of the method (8).

The aim of the study was to determine the limit of detection of IST by the use of samples of intestines experimentally enriched with known numbers of *E.
Echinococcus multilocularis tapeworms and additionally the comparison this method with SCT.

Material and Methods

Estimation of the limit of detection of IST using samples enriched with Echinococcus multilocularis tapeworms. E. multilocularis tapeworms were obtained from the intestines of naturally infected foxes during the examination with the use of SCT (13). Before the experiment began, the collected tapeworms were put into 70% ethanol and stored at 4°C to preserve them against degradation. On the day of the experiment, known number of E. multilocularis tapeworms were put into tubes containing about 5 ml of physiological saline (0.9% NaCl), in order to prepare the doses for experimental enrichment. Twenty four tubes containing 10, 30, 60, and 90 tapeworms (six tubes for each dose) were prepared.

Negative (without Echinococcus) intestines. Negative intestines were selected by the way described previously (8). After 2-week storage at -80°C, the small intestine of foxes were divided into three equal parts (anterior, middle, and posterior). The middle and posterior parts of the intestine were examined with the use of SCT (13). When the Echinococcus tapeworms were not detected in these parts, the anterior part of the intestine was considered as negative. On the other hand, when these tapeworms occurred in the anterior part, they were always observed in posterior parts (9, 16). Overall, 24 of such negative intestines were frozen at -20°C.

Course of the experiment. Twenty four samples with 10, 30, 60, and 90 E. multilocularis worms were examined according to the following procedure. The negative intestines are incised longitudinally. After removing coarse material (stones, bones), E. multilocularis worms, suspended in physiological solution, were put regularly on the whole surface of intestinal mucosa. Then five deep mucosal scraping were taken nearly equal distances from the surface of intestinal mucosa. Adherent materials were transferred to a square plastic Petri dish. Scrapings were squashed between slides and the bottom of Petri dish and examined under a stereomicroscope (15-40x). Detected E. multilocularis worms were counted. Because examination comprised 1/3 part of surface of the intestine, five scrapings (third part of 15 ones required for whole intestine) were collected.

Statistical analysis. The limit of detection (LOD90) - the lowest number of parasites, which can be detected in the samples without precise determination of their quantity - was estimated as the level of worms at which 50% of the replicates are positive (10). All the data were analysed by the use of Microsoft Excel 2003.

The efficacy of IST in examination of naturally infected intestines in comparison with SCT. For that reason, 127 foxes’ intestinal samples (100 from Kielce and 27 from Lublin provinces) were examined by IST and SCT. Firstly, according to the IST procedure (13) mucosal scrapings were taken from the small intestine. After taking the scrapings, preparation and examination of each intestine were continued by SCT procedures (13). Prepared samples were examined under a stereomicroscope (15-40x). Detected E. multilocularis worms were counted.

Results

The limit of detection was estimated as 30 E. multilocularis tapeworms per intestine (for 50% probability of obtaining positive results). None positive samples were found on 3 times lower content of tapeworms (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results of examination of intestinal samples (enriched with different number of E. multilocularis) with the use of intestinal scraping technique (IST)</td>
</tr>
<tr>
<td>Number of worms per sample</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>90</td>
</tr>
</tbody>
</table>
Among 127 samples examined with the use of two methods, eight samples (6.3%) were positive by SCT and only two (1.6%) when IST was used (Table 2). Moreover, in samples (B and C) positive in both methods, the number of tapeworms detected by IST was several dozen times less than by SCT. Numbers of tapeworms found by IST were 4.7% (77 in sample B) and 1.2% (11 in sample C) of respective numbers obtained in these samples by SCT (1,630 and 900). All positive samples found by SCT in Kielce Province were negative in IST.

Discussion

The sensitivity of the IST was earlier estimated by comparison to the other methods. Hofer et al. (7) obtained the 78% sensitivity of IST in relation to SCT. Whereas, Dinkel et al. (5 with the use of reciprocal calculation (based on 165 PCR-positive samples) estimated the sensitivity of the method as 76%, in comparison to the nested PCR. The efficacy of IST in relation to the real number of worms in the intestine was assessed for the first time. The obtained results revealed a relatively low efficacy of IST in echinococcosis diagnostics, especially in comparison with SCT, where LOD95 equaled 10 tapeworms per sample (8). Moreover, the investigation proved that mean numbers of Echinococcus found by IST were several dozen times lower than the real content of these tapeworms in samples (on average only 2% to 3.2% of worms were recovered). That was mainly caused by the method of sampling used in the technique. Namely, scrapings could have ranged only a part of the intestinal mucosa surface and much of material has been left unexamined. Another important reason of a low level of worms recovery were the difficulties connected with microscopic examination - mucus pressed between slide and bottom of Petri dish can significantly decrease the visibility and does not allow to make a correct identification. Obviously, the carried out investigation with enriched samples was only the experimental model and it did not fully represent the examination of naturally infected intestines. Detection of Echinococcus worms from naturally infected intestines is probably even more difficult than in the experimentally enriched samples what has been confirmed by the obtained results in naturally infected foxes.

A preliminary investigation with the use of two diagnostic methods in order to examine naturally infected foxes showed significantly lower efficacy of IST compared with SCT. The IST detected Echinococcus infection only in intestines with a high intensity (1,630 and 900 tapeworms found in SCT). Samples with smaller number of tapeworms were determined in IST as negative. Surprisingly, IST did not detect tapeworms in sample with the highest number of parasites detected by SCT (20,000 tapeworms). Probably, it might result from not sufficiently transparent mucosal scraping, which made a microscopic detection of the tapeworms impossible. It should not be surprising that SCT showed better efficacy. This method was regarded as the “gold standard” (6). Moreover, numbers of tapeworms detected by IST constituted only the slight percentages of numbers obtained in the same samples by SCT: in case of sample B there were 77 tapeworms - what gives only 4.7% of tapeworms found in this sample by SCT (1,630) and in the sample C it was only 1.2% of tapeworms. Additionally, when calculating the limitations of SCT in the analysis (8), the percentage of worms recovered by IST in relation to their expected real amount in naturally infected intestine would be several times lower. IST is only recommended as a qualitative method but such ineffective isolation of tapeworms can also influence on the qualitative (absent/present) results, especially when animals with a relatively low intensity of invasion were examined.

It must be stressed that all Echinococcus positive foxes from Kielce province were found only by SCT. These foxes were negative in IST. Therefore, if the prevalence investigation had been carried out only with the use of IST, the prevalence of E. multilocularis in foxes in this province should have been estimated as 0% (3.6% obtained by SCT examination).

A relatively high limit of detection estimated experimentally in the first part of the study, as well as, the results obtained in field investigations showed clearly that IST method could significantly decrease the results of the examination. That could be hazardous to achieve reliable results of the prevalence studies, especially carried out in the regions with a very low prevalence of E. multilocularis or in the

---

Table 2

<table>
<thead>
<tr>
<th>Technique</th>
<th>Number of Echinococcus tapeworms detected in samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (S)</td>
</tr>
<tr>
<td>IST</td>
<td>0</td>
</tr>
<tr>
<td>SCT</td>
<td>20,000</td>
</tr>
</tbody>
</table>

(L) - sample from Lublin Province; (S) - sample from Kielce Province
countries/regions, willing to reveal that they are free from the parasite.

References