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Method for Determining the Viability of Echinococcus Germ Cells in an in Vitro Experiment

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Abstract Echinococcosis of the liver in children remains an urgent problem in endemic regions, including Uzbekistan, with a high incidence of postoperative recurrences. Prevention of recurrences requires effective intraoperative antiparasitic treatment of the residual cyst cavity. The article presents a new method of express diagnostics of echinococcal protoscolex viability based on the assessment of cuticular layer motility and morphostructure of parenchyma. The method allows reducing the diagnostic time to 1-2 min, increasing the safety of echinococcectomy and reducing the risk of recurrences.

Keywords Echinococcosis, Children, Echinococcectomy, Protoscolexes, Antiparasitic treatment, Rapid diagnosis

1. Relevance

To date, the main treatment option for hepatic echinococcosis in children is organ-preserving echinocolectomy [1]. However, the frequency of postoperative recurrences, according to different authors, remains high 17 -41% [2,3]. In order to prevent recurrences of the disease during surgery, the residual cavity of the echinococcal cyst is treated with germicidal drugs, but the effectiveness of many of them has not been proven to date and is questionable [4]. To assess the effectiveness of antiparasitic treatment, it is important to determine the viability of echinococcal germ cells, which is associated with certain difficulties [5].

In addition, existing methods for assessing the viability of protoscolexes often require considerable time, which increases the duration of surgery and may affect its outcome [6]. In endemic regions such as Uzbekistan, where echinococcosis remains a common disease, the development of a rapid and reliable method of intraoperative diagnosis is a priority to improve the effectiveness of surgical treatment and reduce the risk of recurrence [7].

Purpose of the research: development of a fast and reliable method of intraoperative express-diagnostics of antiparasitic treatment efficiency of echinococcosis germinal elements.

2. Materials and Methods

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The material for in vitro studies were germinal elements of cystic echinococcosis obtained during surgery - echinococcectomy in children with hepatic echinococcosis who were hospitalized at the Specialized Children's Surgical Clinic of SamSMU.

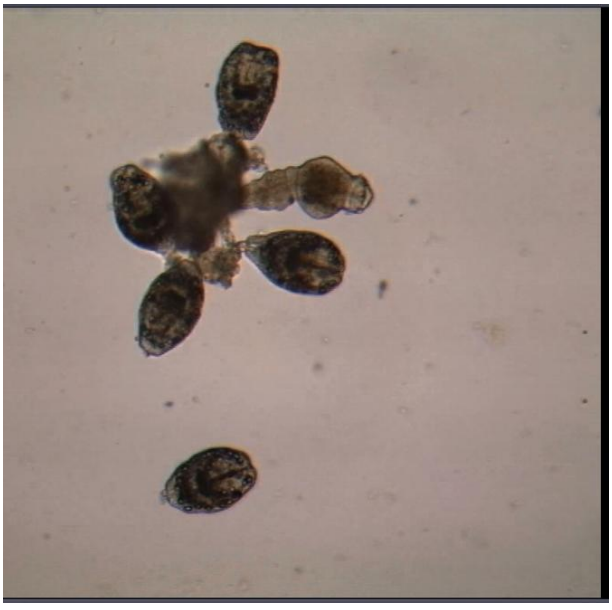

Figure 1. Protoscolexes unerupted and erupted, alive. Before antiparasitic treatment. Zoom 10×10

Earlier, a method for assessing the viability of protoscolexes during echinococcectomy in children was developed in the clinic (invention patent No. IDP 05334). The method is based on the assessment of the motor activity of echinococcal protoscolexes - the ability of protoscolexes to fold and evert [8]. When folding, protoscolexes acquire

an oval shape; when eversion takes place, they acquire the shape of a fungus (Fig. 1). The essence of the technique: after treatment of the echinococcal cyst and fibrous capsule with a germicidal drug, a flush is taken for sampling. In order to activate the motor function of protoscolexes, the sample is placed in nutrient medium at pH 7.5, incubated in the thermostat for 10 minutes, then microscoped. In the absence of motor activity, reduction in size, deformation and brightening of the protoscolex parenchyma, the loss of their viability is diagnosed (Fig. 2).

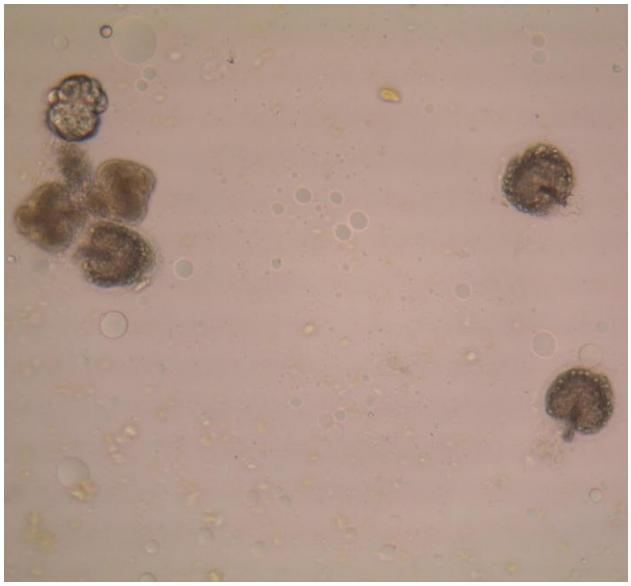

Figure 2. Protoscolexes unerupted and erupted, dead. After anti-parasitic treatment. Zoom 10×10

Using this method in our clinic, we conducted a study of the sensitivity of protoscolexes to various germicidal preparations, on the basis of which we compiled a table of the time of destructive effect on the germ elements of echinococcus.

By conducting further studies, we were convinced that both everted and coiled protoscolexes can be dead or alive. In addition, the ability of protoscolexes to evert and coagulate takes at least 3 to 5 minutes of time, even when motor function is activated by creating favorable conditions (nutrient medium with pH 7.5, incubation in a thermostat at 37.0 for 10 minutes) (Fig. 3). Normally, viable protoscolexes repeatedly evert (a) and coagulate (c) within 30-60 minutes when observed under the microscope.

The above method of assessing the viability of echinococcosis germinal elements after exposure to germicidal drugs requires a long time to perform - 20-30 min, which lengthens the duration of the operation and makes its intraoperative application inconvenient [9].

In order to increase the reliability and reduce the testing time, we developed a new method to determine the viability of protoscolexa. The method is based on the assessment of cuticular layer and parenchyma motility in the form of insignificant "breathing movements" and morphostructure of protoscolexes (patent for invention No. IAP 20120244). The essence of the technique: in the course of surgery, after echinococcectomy, antiparasitic treatment of echinococcal cyst and residual cavity a flush is taken, 2 drops of which are placed in the well of the slide with subsequent microscopy under high magnification (10×100). Microscopic examination evaluates the motility of cuticular layer and parenchyma in the form of insignificant "breathing movements" (Fig. 4 a, b, c, d) and normal morphostructure of protoscolexes (Fig. 5).

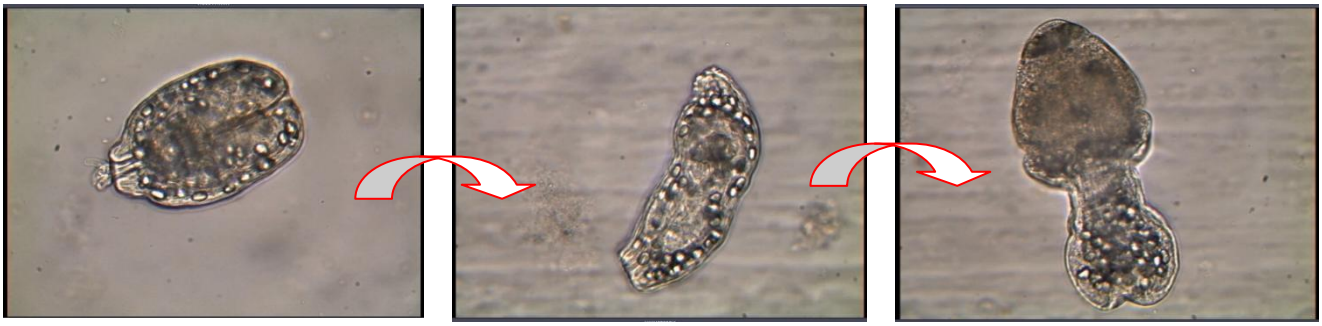

Figure 3. Videophotomicroscopic study of the motor activity of the protoscolex. a-Folded protoscolex; b-Incomplete eversion; c-Full eversion. The duration of the process is 3-5 min

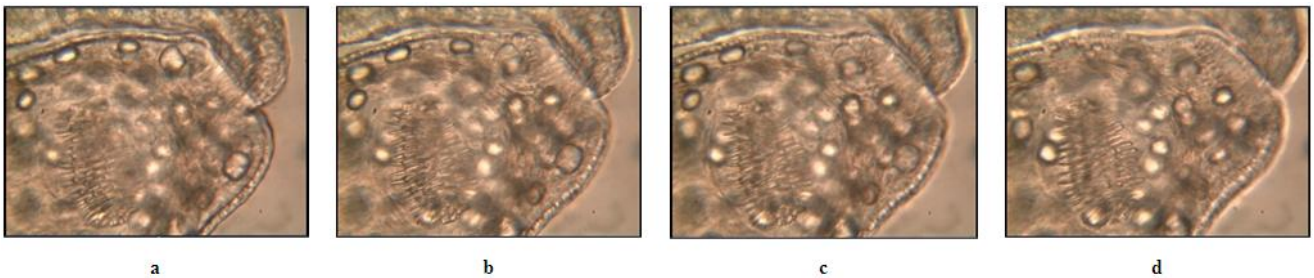

Figure 4. Videophotomicroscopy: on a series of photographs (a, b, c, d) we tried to show the motor activity of the cuticular layer edge and protoscolex parenchyma in the form of insignificant "breathing" movements

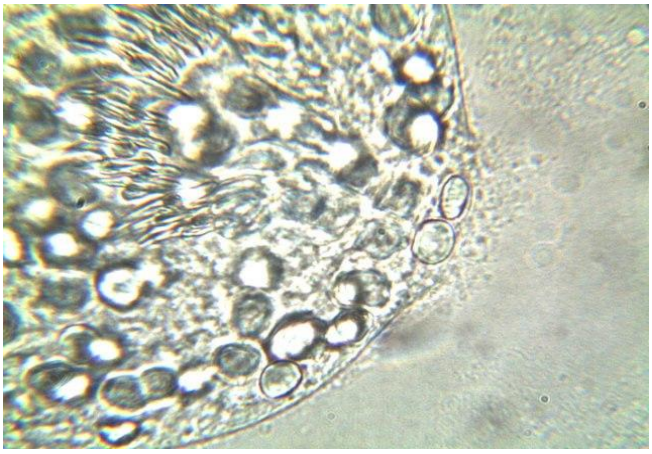

Figure 5. Live protoscolex. Prior to antiparasitic treatment. Zoom 10×100. Preservation of clear structure of parenchyma is noted

3. Results

Complete loss of motility of cuticular layer and parenchyma of protoscolexes, destruction of structural elements and smoothing of parenchyma indicate irreversible destructive changes and death of germinal elements of the parasite (Fig. 6).

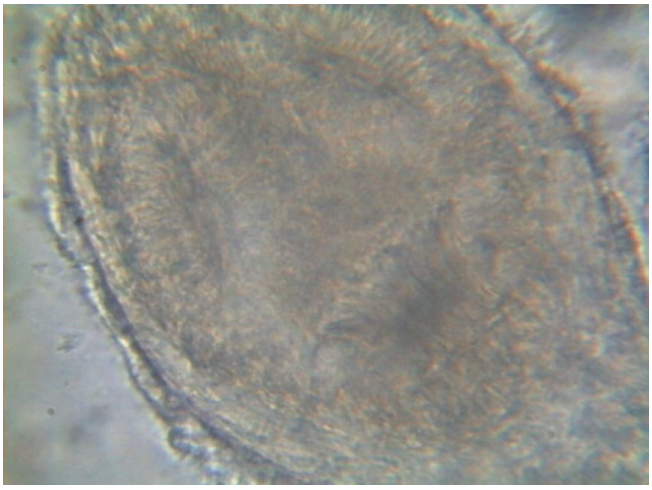

Figure 6. Deceased protoscolex after antiparasitic treatment. Zoom 10×100. Complete absence of motor activity of cuticular layer and parenchyma, smoothness of parenchyma structure, margins of protoscolex are festooned

The developed new method for determining the viability of Echinococcus protoscolexes does not require incubation in special nutrient media, incubation in a thermostat and long-term observation under a microscope of functional activity (ability to fold and evert), which significantly reduces the study time. This technique requires 1-2 minutes to determine viability. Under high magnification of the microscope (10×100), the normal morphostructure of the protoscolex parenchyma is clearly defined, and the phenomenon of motility of the cuticular layer and parenchyma in the form of insignificant and low-amplitude “breathing movements”, clearly defined in living protoscolexes, is a well-differentiated sign of viability. In dead protoscolexes, the signs of destruction of structural elements and smoothing of parenchyma are clearly distinguishable, the movement of cuticular layer and parenchyma in the form of insignificant respiratory motility is completely absent.

4. Discussion

Determination of the above features is simple and does not require a specially trained laboratory parasitologist. Comparison with the previous method based on the assessment of the ability of protoscolexes to curl and evert shows that the new method is significantly faster (1-2 minutes vs. 20-30 minutes) and does not require complex sample preparation [10]. This makes it more convenient for intraoperative application, which is especially important when time is limited during surgery.

The effectiveness of antiparasitic treatment has a direct impact on reducing the risk of recurrence of echinococcosis, and our method can promptly confirm the death of protoscolexes, which increases the safety of surgery [11]. Unlike other methods such as staining of protoscolexes or the use of biochemical markers, our approach does not require additional reagents and sophisticated equipment, making it affordable to apply [12].

5. Conclusions

Thus, the developed new method of determining the viability of Echinococcus protoscolexes after treatment of the echinococcal cyst and fibrous capsule cavity with an antiparasitic agent of contact action is reliable, fast and simple, which is important in echinococcosis surgery.

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Conflicts of interest. The authors have no conflicts of interest.

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