PRACTICAL EXPERIENCE IN USING THE MINI PARASEP FAECAL PARASITE CONCENTRATOR FOR CRYPTOSPORIDIUM SPP. OOCYSTS DETECTION IN STOOL

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Introduction

Cryptosporidiosis (A07.2, MKX-10/ICD-10) is an insufficiently studied protozoan disease that is caused by the protozoans of the genus *Cryptosporidium* (type *Apicomplexa*) that are capable of invasion, reproduction and parasitism inside the enterocytes of the gut tract mucosal filli (mainly in the small intestine). The causative agents lead to the development of specific infectious process with the characteristic clinical symptoms, such as "watery diarrhea" [1, 2].

At present, f among the 27 "recognized" cryptosporidium species, the ability to cause the disease in humans is proven for nearly 20. But the majority of the cases (more than 90 %) registered in humans worldwide were caused by two species - *C. hominis* і *C. parvum* [3, 4].

The efficacy of prophylaxis and treatment of cryptosporidiosis is based on the timely and quality diagnostics, which allows recognizing the disease incidents actively, prevents the development of the heavier symptoms, and limits the spreading of the parasites. Cryptosporidiosis as a diagnosis is established based on the epidemiological and clinical data, as well as results of laboratory tests [5, 6]. The latter play a considerable role during verification of cryptospridiosis etiology considering the polyetiological nature of the disease and the similarity of its clinical manifestations to other diarrheal infections of viral, bacterial or parasitic etiology. In order to diagnose this parasitosis, the methods of three groups are the applied most often: microscopic, immunological and molecular genetic [3, 6, 7].

Microscopic methods remain the most popular in the regular laboratory diagnostics and are based on the determination of the parasitic oocysts (sometimes other parasitic cycle stages, such as meronts, merosoits or sporozoits) in the smears of the research material (mainly fecal, sometimes sputum, bronchial mucus, etc.), which for today is considered as "gold standard" of this parasitosis etiology [1, 3, 7, 8]. There are multiple variants of microscopic cryptosporidia determination, but for the present no universal method was developed that would be always providing stable and sufficient efficacy for obtaining univocal test results (positive or negative) [6-9]. The well-known drawback of the microscopic diagnostic methods of cryptosporidiosis is their relatively low sensitivity. ($\geq 10^4$ oocysts/g) [2, 3, 4]. Therefore, the researchers are actively developing methods of enrichment of the research material (the increase in the oocysts` concentration), which not only provides higher productivity of the oocysts` determination, but also simplifies and shortens the research protocol and increases the level of biological safety [2, 9, 10].

Among the large quantity of the routine methods of purification and concentration of oocysts for the increase of cryptosporidiosis diagnosis efficacy the method of centrifugal sedimentation in the formaline ethylacetate mix (FECS) is considered to be the most effective [6, 9, 12]. The FECS method is characterized by a number of advantages compared with other purification methods: it is simple to recreate, it can be used for determination of agents of other intestinal protozoan diseases; it also provides the means to preserve important morphologic features of the search objects, etc. But the FECS method has a number of significant drawbacks: it requires the presence of additional preliminary procedure – the filtration of fecal samples suspensions in order to eliminate the big-sized non-digested food particles that could hide cryptosporidia oocysts or other parasites; insufficient level of validity of the method; significant labor intensity and material expenditure of the procedures, as well as a high level of possible inside infection of the laboratory workers due to the direct contact with the infected samples and aerosol. In order to eliminated the significant drawbacks of the traditional version of the FECS method, the scientists residing abroad, as well as commercial providers have proposed several approaches to its optimization, that are based on the use of special single-use systems for determination of eggs, larvae and cysts of helminthes, as well as protozoan oocysts in the fecal samples by concentration of the former with the help of centrifugation and filtration through special filters inside a closed system [13, 14]. In the latter years, different types of systems are actively used (Mini, Midi, Maxi, with reagents solutions and without them), including Parasep® Faecal Parasite Concentrators produced by Apacor Ltd./DiaSys Europe Ltd. [13-15]. Until present times, a significant scientific and practical experience of application of Parasep® concentrators for diagnosis of more than 20 parasitic diseases: opistorchosis, fascilosis, dicrociliosis, metagonimosis, nanophietosis, diphylolepidosis, himenoledpidosis, ascaridosis, ankilostomosis, strongiloidosis, nekotorosis, lyambliosis and others was accumulated [14-17]. But there are no distinct and convincing data that would have determined certain technological features of the application of Parasep® type concentrators for cryptosporidiosis diagnostics [10, 18]. In the studies that have used Parasep® concentrators, high variability of the procedures involving the use of the latter (often the differences are related to the amount of the fecal sample, centrifugation regimes, the composition of the liquid sedimentation system, etc.) are observed.

The aim of the study

The evaluation of efficacy of the Mini Parasep® Solvent Free Faecal Parasite Concentrator compared to the traditional method of concentration (centrifugal sedimentation in the formalin – ethyl acetate mix) during the testing of the clinical material (fecal) for the presence of the oocysts of the parasite *Cryptosporidium* spp.

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Materials and Methods

The object of this study were the 102 fecal samples obtained from children aged from 1 month to 17 years, that were provided with medical assistance at the infection department of the № 2 Community Medical Establishment "Children territorial medical association " (Kramatorsk) for different intestinal infections that were accompanied by acute diarrhea. The fecal samples arrived at the laboratory with special plastic containers with a conservant - 10,0 % water formalin solution (FS) at the volume ratio (v:v) (1:1).

The stages of concentration of the oocysts from the fecal samples under the influence of centrifugal sedimentation in the formalin-ethyl-acetate mix (FECS) are shown in the table 1.

Table 1. Main stages of the purification and concentration of *Cryptosporidium* spp. oocysts with the help of modified FECS method

Stages, №	Specifications of the sedimentation system and other applied methods	Procedures and their short description
1	2	3
1	Container with the fecal sample, centrifugal test tube 10,0 ml, cold (t^o = 4±0,5 °C) 10,0 % v/v FS	*Preparation of the primary fecal homogenate in the FS*: put a fecal sample of around 1,0 g/ml of mass/volume into the test tube with пробірку 3,0 ml FS; homogenize thoroughly
2	A 50 ml beaker or a glass with a spout, glass conical bailer 55 – 70 mm in diameter, filter composed of 2-4 gauze layers (with the square pore side size around 800 μm)	*Elimination big-sized conglomerates from the fecal homogenate through filtration*: pour the fecal homogenate through the gauze filter into the beaker with the help of the bailer
3	Calibrated centrifugal test tube, 20 ml and 2 calibrated glass pipettes, 5 ml and 10,0 ml, rubber pear-dropper, rubber conical cork 14,5 mm in diameter, cold FS, liquid de-oiling agent (ethyl acetate, EA)	*Formation of the required sedimentation system*: pour the filtered fecal sample into the test tube and add around 4,0 ml of FS and 3,0 ml EA (the general volume around 10,0 ml); close the tube by the rubber cork and thoroughly suspend the mix during 30 sec by horizontal shaking of the tube
4	Laboratory centrifuge, balance scales, calibrated glass pipette for 1,0 or 2,0 ml, rubber pear-dropper, cold FS, paper napkin, paraffin film	*Sedimentation of the test oocysts*: balance the centrifugal test tubes with the fecal suspensions by dose-dependent FS addition; cover the tubes with paraffin; centrifuge the tubes at 1100 g for 3 minutes
5	Vial tray, clean glass stick (single-use wooden or plastic toothpick), calibrated glass pipette, 1,0 ml and rubber pear-dropper or pipette dosator with regulated volume from 1 to 10 μl and single-use plastic and corresponding plastic chip, clean/disinfected glass slide (25×75×2 mm), liquid fixer (ethanol 96 %)	*Extraction of the sedimented homogenate and preparation of the smear from the microscopic study*: carefully place the tube onto the tray; with glass stick or a toothpick separate the oil cork from the inner wall of the tube; extract the oil cork and liquid supernatant, bending the tube over; re-suspend the sediment in the tube with the pipette (or with the tipped dosator); extract around 5 μl homogenate; prepare the round-shape smear on the glass (around 2 cm in diameter); dry the smear thoroughly (30 minutes or more) and fix with the liberal administration of the fixer (the duration of the fixing is around 3 minutes or more)

Mini Parasep® Solvent Free Facal Parasite Concentrator (further - Parasep®) ("Apacor Ltd." , Unit 5 Sapphire Centre, Fishponds Road, Wokingham, Berkshire RG41 2QL, England (https://us.vwr.com/.../parasep-fecal-parasite-concentrators) (Figure 1), that was tested for the cryptosporidia oocysts determination is a collapsible plastic test tube (around 1,5 cm in diameter and 15 cm in length) that is composed of 4 main components:

- a homogenization camera (a base tube) for the fecal samples (contains 2,4 ml of the water 10,0 % solution of formaline (FS), 0,9 ml ethylacetate and 0,05 ml tritone X-100);
- a spoon for the sample extraction with the filter handle;
- conical container for the accumulation of the filtered material (sedimentation recipient container);
- separate twisting cork for the base container (before the input of the fecal sample);
- and conical container for the filtered sediment, obtained as a result of the centrifugal sedimentation.

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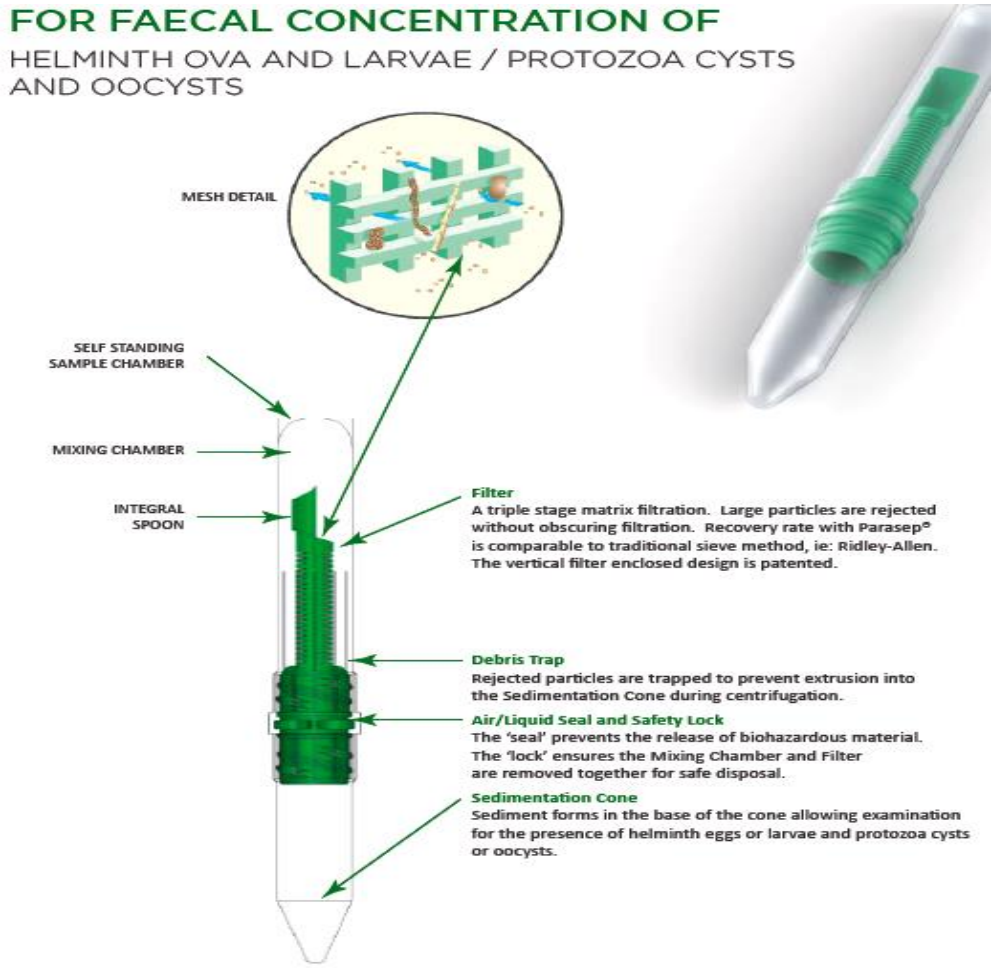

Figure 1. The outer appearance, main components, and structural composition of the Mini Parasep® Solvent Free Faecal Parasite Concentrator (https://www.apacor.com/wp-content/uploads/2017/09/APA175-Mini - Parasep-SF-EU-Protocol-v3.0-2017.09.pdf)

During the topic study with the use of Parasep® concentrators we were trying to act according the protocol and manual from the manufacturer ("Apacor Ltd."©, 2017. Mini Parasep® SF-EU Protocol (http://www.apacor.com/products/parasep-sf-eu-protocol/) [19].

In table 2 the stages of purification and concentration of the cryptosporidia oocysts in the fecal samples with the use of Parasep® concentrators are listed.

Table 2 . Procedures of the purification and concentration of the *Cryptosporidium* spp. oocysts with the Parasep method

Procedure stage, №	Procedure and its short description
1	*Preparation of the sedimentation system, the extraction and homogenization of the fecal sample*: detach the spoon with the filter handle from the base tube (homogenization camera); add to the sedimentation solution (contains 2,4 ml water 10,0 % (v/v) formalin solution /SF and 0,05 ml triton X-100) 0,9 ml of ethyl acetate /EA); with the help of the spoon take up an aliquot of the fecal sample of the mass/volume around 1,0 g/ml (two full spoons), put into the base tube and thoroughly homogenize with the sedimentation system
2	*Assembly of the Parasep® concentrator for the centrifugal sedimentation for oocysts determination*: assemble the homogenization camera together with the spoon with filter handle and conical container for the filtered material; additionally suspend the mix for 30 seconds by horizontal shaking of the Parasep® concentrator
3	*Sedimentation of the oocysts by centrifugation:* Turn the full Parasep® concentrator so that the conical container is down; centrifuge at 1100 g for 3 minutes.
4	*Extraction of the sediment homogenate and production of the smear for the microscopic study*: carefully take the Parasep® concentrator from the centrifuge; holding the latter upright, separate the conical container with the filtered material; extract the oil cork and liquid supernatant, homogenize the sediment and prepare the smear thereof

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The study of the fecal samples with the help of FECS and Parasep® methods was carried out by three different researchers with stage-specific timing of the each procedure (see. Procedure stage, Tables 1 and 2).

The following reagents and equipment were used in course of the study: formalin "37 %" («Novochim», Ltd., Ukraine), ethyl acetate (EA) "99,7 %" ("Chimreserve", Ltd., Ukraine), alcohols, solutions of pigments and decolyzers - "Dubov`yzivskiy Alcohol Plant", Ukraine, OJSC "Shostka Plant of Chemical Reagents", Ukraine); medical centrifuge OPn-3.02 (transnational corporation "Dastan", Kyrgyzstan); microscope "MICMED-2" Yu-33.22.926 (OJSC "LOMO", Russian Federation) (×1500). For the comparison of the productivity of the purification and concentration of cryptosporidia oocysts and other characteristics of the FECS and Parasep® methods fixated smears (ethanol, 96 % (v/v) were stained by the modified (cold) Ziehl-Neelsen staining method (mZN) [3, 11]. During the microscopic study of the stained smears from the enriched fecal sediment for the evaluation of the presence (with determination of the qualitative and quantitative parameters) and cryptosporidia identification (based on the evaluation of the shape, size, typical inner structure) the criteria recommended by the foreign scientists were used [9]. The following parameters were determined: 1) Cryptosporidia oocysts presence was determined according to the rules: «-» - no oocysts found; «+» - less than 5 oocysts found in the smear; «++» - 1 to 10 oocysts found in the field of vision of the microscope; «+++» - 11 oocysts or more determined in the field of vision of the microscope (total enlargement ×400, dry or water immersion). Also, total oocyst count was carried out (the total number of oocysts - **TNO**) in the smears produced from the 5 μl enriched sediment of the positive fecal samples (total microscope enlargement ×1000, oil immersion); 2) Cryptosporidia oocysts shape (in species *C. hominis* and *C. parvum* shape is round with the thick wall that is distinctly visualized during microscopy (total enlargement ×1000, oil immersion); 3) oocysts size, i.e. diameter (**DO**): in species *C. hominis* and *C. parvum* he size ranges from 4 to 6 μm (most often - 4,5×5,5 μm). Determination of these parameters is a diagnostically important task as many other *Cryptosporidium* species have different oocysts sizes (for example, in *C. muris* – 5,5×7,5 μm, and in *C. galli* – 8,5×6,4 μm). Morphometric studies with DO determination were carried out in the 10 oocysts of every positive fecal sample (total enlargement ×1500, oil immersion, changeable ocular scale);

4) Presence of the characteristic internal structure of the cryptosporidia oocysts (**ISO**), that is the main identification parameter (for differentiation of the oocysts from the morphologically similar cells of yeasts and yeast-like fungi, non-digested pollen remains or nuclear shells of the plant cells, etc.), that is determined by the presence of four intraoocyst sporozoits;

5) Probability of the concealment of the oocysts - **PCO**, that was determined by the count of the total quantity of big-sized conglomerates with visually evaluated surface area that is ≥ 1/5 of the microscope field of vision (approximately3000 μm ^{2}) in the fecal smears produced from the 5 μl of the enriched fecal samples; The studied macro- and microscopic objects in the produced smears were fotographed by digital camera "Olympus C7070 Wide Zoom" ("Olympus", Japan). Statistical evaluation of the obtained experimental data was carried out according to the rules of the rank-and-file and alternative variable statistics with the help of program package STATISTICA 10 (Microsoft Office Excel-2003).

Results and discussion

In the studied 102 fecal samples obtained from children by parallel application of both method s(FECS i Parasep®) complete coincidence (r_ϕ=1) in qualitative determination/non-determination of the cryptosporidia in the research samples was established. The results of comparison of efficacy of the purification and concentration of the cryptosporidia oocysts with the help of FECS and Parasep ® methods is presented in the table 3 (monotype values that characterize the FECS and Parasep® methods were not included in the table). Oocysts were found in 4 samples (3,9 % from total quantity) (figure 3), that was in the range of this parameter in the same group of increased risk in the developed countries (from 1,4 to 4,1 %) [1, 3, 5, 6].

Table 3 Results of the comparison of the efficacy of purification and concentration of cryptosporidia oocysts with the help of FECS and Parasep methods

№, п/п	Main criteria that characterize the methods	Method	
		FECS	Parasep®
1	Approximate cost of one test, Hrn.	28-32	59-60
2	Material cost of one test *	5	1
3	Labor intensity *, min.	9,8 - 14,5	4,5 - 5,5
4	Validity*	1	3
5	Bio-safety*	1	4
6	Productivity, TNO*	1,0	1,5
7	Probability of the oocyst concealment, PCO***	1,0	3,8

Remarks: * - rating index was determined according to the accepted conditional scale from 1 to 5 with general evaluation of the latter: "low" - 1 and 2, " moderate" - 3 and "high" – 4 and 5; ** - TNO – is an abstract parameter of total number of oocysts. The conditional value of TNO for the FECS method is accepted at 1, 0; *** - PCO – abstract parameter of probability concealment of the oocysts. Conditionally, for the FECS method the PCO value is accepted at 1, 0.

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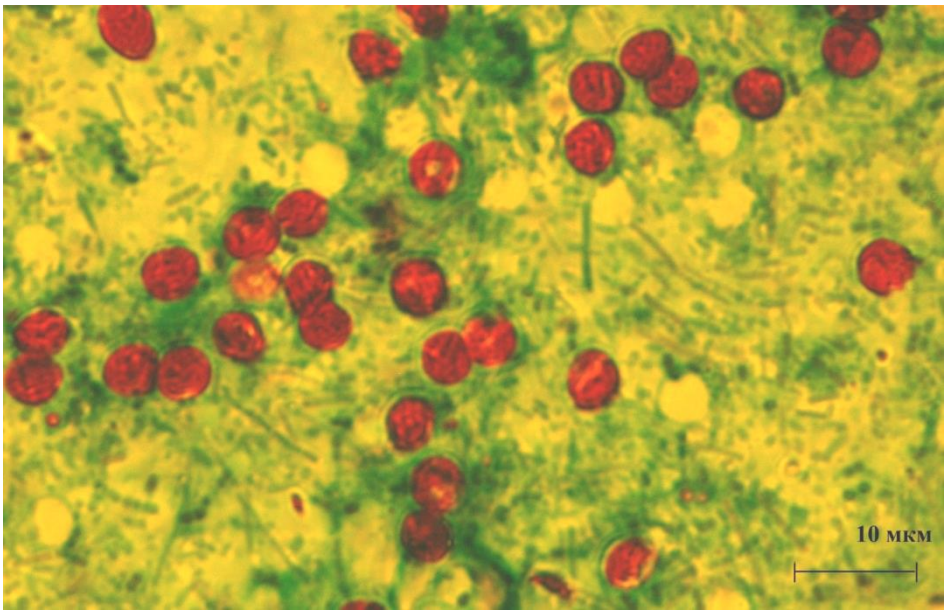

Figure 3. The preparation of the smear of the enriched Parasep fecal sample, staining mZN (cryptosporidia oocysts are stained in different shades of bright red color on green background of the background stain of the green malachite (light microscopy, total enlargement ×1000, oil immersion).

In the total cost of the purification and concentration of cryptosporidia oocysts with the FECS and Parasep® methods total cost of the applied methods consituted 44,0-50,0 % and 87,5-89,0 % respectively, and the cost of the specialist labor – 50,0-56,0 % and 11,0-12,5 %, respectively. On the contrary, in the economically developed countries during the application of Parasep® method the reverse relationship is observed concerning the costs of applied methods and specialist labor (around 20-25 and 75-80 %, respectively), which is explained both by the significantly higher salary of the medical personnel labor and the lower cost rate of the fecal parasitological concentrators of the Parasep® type.

The obvious advantage of the Parasep® method compared to the FECS method is the presence of the full set in the fecal parasitological concentrator itself. In comparison, the FECS method has a high material costs and requires 10-15 units of the different types of laboratory utensils, additional equipment, reagents, etc. (table 1). Therefore, based on the rating 5-grading scale of the material cost, one FECS test is considered to have a high cost, whereas Parasep® method is of a low cost.

The other advantaged of the Parasep® method is its significantly lower labor cost (2,2-2,6 lower) compared to the FECS method, that can be expressed in the minutes of the time spent by the specialist for one test. The results of our chronometric duration of the stages of the fecal samples enrichment procedure almost fully coincide with the data of the similar experiments carried out by the USA specialists; the exception is the duration of centrifugation stage (according to our protocol - 3 minutes, and according to the USA protocol – 1 minute) [13].

The application of the single-type fecal parasitological concentrators Mini Parasep® SF, protocol adherence and target use according to the user manual provides a moderate level of validity of the Parasep® (variation of the fecal quantity and volume of the concentrated sediment in the tube end), whereas the FECS method compared to the previous one is characterized by low rating parameter of validity. At present, Parasep® method is considered as a base for standardization of the fecal parasites concentration procedure (eggs and larvae of the helminthes, cysts and oocysts of the protozoans) [10].

Compared to the FECS method, the doubtless advantage of the Parasep® method is its high biosafety rating, due to the elimination of the possibility of the contact of the lab personnel with the infected fecal sample and the decrease in the contamination risk of environment contamination by the former [13, 19].

Considering the general efficacy between FECS and Parasep ® methods, the most important rating criteria were: the parameter of the quality determination/non-determination of the cryptosporidia oocysts, abstract parameter of the total quantity of the determined oocysts – TNO and abstract parameter of the probability of the oocysts concealment - PCO (see table 3). During the carrying out of the experiments for the determination of the mentioned above parameters the universal methodical approaches of preparation production (from the 5 µl of sediment), staining (mZN method) and microscopic determination and count of the oocysts (with the help of light microscopy with total enlargement ×1000).

During analysis of other results of our experiments, a significant variation in the quantity of determined oocysts was established in smears produced from different positive (n=4) fecal samples – from 947 to 3952 with the help of FECS method and from 1300 to 4800 with the help of Parasep ® method, which indicates the significant influence of subjective factor on the result of the counting (the last varied in some smears according to different specialists, at 19,1 - 32,5 %). Such circumstances also influence the data of the determination and counting of the quantity of the big-sized conglomerates, that could conceal the cryprosporidia oocysts (variation of their total

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quantity was observed in different samples from 13 to 232, and the influence of the subjective factor sometimes reached 18,4 - 23,7 %). It explains the inexpediency of representation of the test results of such studies in the form of a range of each measured absolute value, their total group value or their log value and substantiated the logic of the widespread approach among the foreign scientists – the application of certain abstract parameters. In this case, the values of the abstract parameters of one of the compared methods (well-known, as a rule) is accepted as 1,0 (or 1+), and the value of the parameter of a different method is relative towards the former value, which allows to demonstrate, determine and evaluate the advantages and disadvantages of each of the methods compared [14, 18, 19]. As seen from the data of the table 3, the determined value of the parameters TNO and PCO, which, respectively characterize the productivity of the determination of cryptosporidia oocysts in the enriched sediment and probability of their concealment by the present big-sized conglomerates is relatively bigger in case of Parasep® method (1,5 and 3,8) compared to the FECS method, for which the value of both parameters was accepted as 1,0.

In course of paired comparisons of the results of determination in the positive fecal samples of the total cryptosporidia oocysts count as well as the big-sized conglomerates (n=4) the differences in these results obtained by the FECS and Parasep® methods reaches the statistical credibility ($p \leq 0,05$). In this the bigger value of the TNO parameters is the positive characteristics of the Parasep® method, whereas the bigger value of the PCO is on the contrary the negative characteristics of this method, which indicates its drawbacks. The latter is explained by the relatively bigger surface of the pores (near $1,8 \times 10^5$ μm^2) of the original mesh of the Parasep® filter, that allows the appearance of both the big-sized eggs, larvae and helminth trophozoits, as well as a significant quantity of conglomerates that can conceal the lesser cysts and oocysts of the protozoans. The results of the experiments recreated by the FECS method using the 2-4 layers of gauze as the filter (with the side size of the square pore around 800 μm), demonstrated the quantitative decrease of both the cryptosporidia oocysts and big-sized conglomerates.

Despite the mentioned above drawbacks of the application of the Parasep® concentrator (the increase of the total cost of the research almost twice and increase 3,8 times the quantity of the big sized conglomerates that can potentially conceal the forms of parasites to be found) the present method has a number of rating advantages compared to the traditional FECS variant: the low material cost and significantly lower (2,2-2,6 times) labor costs, moderate validity level, high bio-safety and higher (1,5 times more) productivity of the microscopic determination of the total cryptosporidia oocysts count.

Conclusion

As a whole, the observed advantages and disadvantages of the *Cryptosporidium* oocysts purification and concentration procedures during application of the FECS and Mini Parasep® SF - EU Facal Parasite Concentrator do not contradict the data of the foreign specialists. It is appropriate to optimize the oocyst` concentration and purification procedures applied for the detection of the former in the fecal samples with the help of microscopy by application of the fecal parasitological concentrator “Mini Parasep® Facal Parasite Concentrator” produced by “Apacor Ltd.”

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PRACTICAL EXPERIENCE IN USING THE MINI PARASEP SOLVENT- FREE FAECAL PARASITE CONCENTRATOR FOR THE *CRYPTOSPORIDIUM* SPP. OOCYSTS DETECTION IN STOOL

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Introduction. Cryptosporidiosis is a protozoan illness caused by the protozoans of genus *Cryptosporidium* (type *Apicomplexa*), that are able to parasitize in the enterocytes of the intestinal mucosa villi, causing a specific infectious process with such manifestations as "watery diarrhea". The efficacy of prophylaxis and treatment of this parasitosis is based on the timely and quality laboratory diagnostics that is most often carried out with the help of microscopic methods. In order to increase the productivity of oocysts determination in the fecal samples different methods of enrichment in the latter are being used. **The aim of the study –** evaluation of the efficacy of application of Mini Parasep® Solvent Free Faecal Parasite Concentrator compared to the traditional method of concentration (centrifugation in the formalin ethyl - acetate mix) in course of focused study of the fecal samples for *Cryptosporidium* spp. oocysts presence. **Materials and methods.** The object of the study were 102 fecal samples (with 10,0 % aqueous formalin at the ratio 1:1) from children with diarrhea aged from one month to 17 years that were provided with regular medical aid. Purification and concentration of cryptosporidia oocysts was carried out with the help of centrifugation in formalin ethyl-acetate mix was carried out according to the widely accepted method. During the application of Mini Parasep® Solvent Free Faecal Parasite Concentrator ("Apacor Ltd.", England) the method recommended by the provider was adhered to, except the sedimentation by centrifugation stage that was carried out at 1100g for 3 minutes instead of 1 minute. Smears were prepared from the supernatant that were stained with the modified (cold) Ziehl-Neelsen staining method. The oocyst purification and concentration procedure was carried out by different specialists with taking into account of each stage chronometry. During microscopy of the stained smears of enriched fecal sediment the following parameters were evaluated: cryptosporidia oocysts presence, size, shape, typical inner structure, as well as the probability of concealment of the oocysts that was calculated by the number of big conglomerates (≥ 1/5 field of vision of the microscope). **Results and discussion.** In the studies 102 fecal samples from children during parallel application of both methods complete coincidence of parameters of quality oocysts determination/non-determination (r_ϕ=1) was established. Oocysts were found in 4 (3,9 %) studied samples that was in the range of this parameter values in similar groups of increased risk in developed world countries. Despite such drawbacks of Mini Parasep® Solvent Free Faecal Parasite Concentrator application as the almost two-fold increase

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in overall study cost and the 3,8 increase in quantity of the big sized conglomerates that could potentially conceal the parasitic types being sought, this method had a number of significant rating advantages compared to the traditional method of centrifugation in formalin ethyl acetate mix: the low material cost and significantly lesser (2,2-2,6 times) labor cost, the moderate validity level, high biosafety and higher (1,5 times) productivity of the microscopic determination of the general quantity of cryptosporidia oocysts.

Conclusions. The advantages and disadvantages of cryptosporidia oocysts purification and concentration procedures in application of traditional variant of centrifugation in formalin ethyl acette mix and in application of Mini Parasep® Solvent Free Faecal Parasite Concentrator determined in course of the study do not contradict the topic data from foreign scientists. It is expedient to optimize the cryptosporidia oocysts purification and concentration procedures for determination of the latter in the fecal samples with the help of microscopy with the help of parasitological concentrator of the Parasep® type.

Keywords: *Cryptosporidium*, Parasep ® Faecal Parasite Concentrator, detection, oocysts.

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