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Original article

CRYPTOSPORIDIOSIS IN CHILDREN – CLINICAL FORMS AND CLINICAL CASES

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ABSTRACT

Cryptosporidiosis is one of the most common causes of diarrhoea worldwide, especially in the vulnerable groups of children between 0-5 years and immunocompromised individuals.

The **purpose** of the study is to estimate the prevalence of the *Cryptosporidium* infectionin children from North-eastern Bulgaria and to determine the specifics of the clinical forms through the detailed description of several clinical cases.

Material/Methods: Stool samples of 458 children were investigated for the presence of *Cryptosporidium* spp. oocysts with specialized modified Ziehl–Neelsen staining and with an immunological test for detection of stool antigens. Prevalence estimates are presented in four groups - aged less than five years with or without symptoms and more than five with or without symptoms.

Results: The overall prevalence of cryptosporidiosis in children from North-eastern Bulgaria is 2.18% with the highest incidence (3.06%) in the age group between 0-5 years with diarrhoea. All different types of infestation of the gastrointestinal tract are observed, from an acute infection that requires hospitalization, through mild or persistent diarrhoea and asymptomatic invasion (in1.53%). For each of the specified forms, the details of the clinical presentation, laboratory and physical findings are presented as separate clinical cases.

Conclusions: Our findings demonstrate that broad-spectrum of clinical forms of *Cryptosporidium*-associated diarrhoea in children exist but up till this study are typically mis- or undiagnosed. The tendency that exists in eastern European countries, including ours, to neglect or underestimate the impact of cryptosporidiosis, is particularly dangerous and should be changed especially in the risk groups of children and immunocompromised adults.

Keywords: *Cryptosporidium*, cryptosporidiosis, cryptosporidiosis in children, clinical forms, diarrhoea

INTRODUCTION

Cryptosporidiosis is a diarrhoeal illness that in humans is most commonly caused by *Cryptosporidium hominis* or *C. parvum*. It has a worldwide distribution facilitated by all routes of the fecal-oral mechanism- human to human, zoonotic, water- and foodborne. The clinical course of cryptosporidiosis includes varying in severity and duration diarrhoea, depending on numerous characteristics of the infected host: age, nutrition, co-infections and immunity [1–5]. The age of the host is the main factor in the pathophysiology of the cryptosporidiosis. The most vulnerable are children between the ages of 0 and 5 years, especially those younger than two years. The immature immune system (especially the mucosal immunity) and the increased chance for environmental exposure lead to a more severe course of cryptosporidiosis in infancy [1, 2].

The last Global Burden of Disease Study (2010-2015) estimates that infectious diarrhoea contributes approximately 1.3 million deaths globally [4,6]. The three leading causes in the most affected group of children up to 5 years are rotavirus enteritis, cryptosporidiosis and shigellosis [4,6]. Recent epidemiological studies using PCR and antigen detection tests show that *Cryptosporidium* spp. is identified in 15–25% of the children with diarrhoea, which renders this parasitosis severely underdiagnosed and probably even more prevalent than was previously estimated [2].

Cryptosporidiosis-associated involvement of the intestinal tract can be classified into several clinical forms. Acute diarrhoea with intoxication and risk of dehydration is mainly observed in newborns and small children (<twoyears).Persistent and chronic diarrhoea can be a significant problem in children with predispositions (malnutrition, comorbidity)or severely immunocompromised individuals.Especially in developing countries, it leads to weight loss and stunning in children's development. Most common are the sporadic cases of mild and self-limited diarrhoea in children of all ages and immune-competent adults. The same groups are the ones where asymptomatic infestation with *Cryptosporidium* spp. is also described [1, 7]. These last two "undetectable" conditions enable the easy spread of the infection among the general population and the other susceptible individuals. In patients with primary or secondary immunosuppression *Cryptosporidium* spp. are commonly identified as opportunistic pathogens causing life-threatening atypical and extra-intestinal forms other than diarrhoea.[1, 3, 5, 8]

The **aim** of this study is to estimate the prevalence of cryptosporidiosis in children from North-eastern Bulgaria in two age groups – between 0-5 years and over 5 years. In addition, we aim to outline the specifics of the different

clinical forms of diarrhoea caused by *Cryptosporidium* spp. in children through the description of several clinical cases.

MATERIAL AND METHODS

A total number of 458 children (age between 0 months up to 18 years) were tested for cryptosporidiosis between 2014 and 2018 in one of the Parasitology laboratories in Northeastern Bulgaria. All patients included in the study submitted at least one stool specimen for morphological identification of intestinal parasites (IP).The specimens were collected in labelled plastic vials, transported to the laboratory and stained or fixed at the day of the collection. To maintain patient confidentiality, only demographic data (sex, age, and residence) was obtained.For the morphological identification of *Cryptosporidium* spp.oocyst, three slides per patient were prepared and stained with the modified Ziehl–Neelsen stain (mZNS) for selective identification of coccidian parasites according to a standardizied staining protocol [9]. In a fraction of the samples (~30%) the morphological examination was aided by immunological detection of stool antigens using enzyme immunoassays (ELISARidascreen® *Cryptosporidium*) or immunochromatographic tests (RIDA® Quick *Cryptosporidium/Giardia* Combi).

Descriptive statistics and prevalence estimates are presented within the 95% confidence intervals.

RESULTS

The outcomes of the morphological and immunological tests for identification of *Cryptosporidium* spp. in children were pooled into single positive or negative result, one for each patient, regardless of the number or type of tests performed.The positive findings in the investigated groups (children ≤5 years with or without symptoms and 5+ with or without symptoms)are presented in Table 1 with the corresponding prevalence estimates. The overall prevalence is 2.18% ± 1.34% with the highest frequency (3.06% ± 2.24%) in children between 0-5 years with diarrhoea. The ratio of infestation with *Cryptosporidium* spp. in asymptomatic children (1.53% ± 2.11%) is another important result.

Table 1. Prevalence of cryptosporidiosis in symptomatic and asymptomatic children in different age groups.

	Symptomatic			Asymptomatic			Total		
	Tested	**/+/**	**Prevalence (% ± CI, p=0.05)**	**Tested**	**/+/**	**Prevalence (% ± CI, p=0.05)**	**Tested**	**/+/**	**Prevalence (% ± CI, p=0.05)**
Children 0-5 y.	229	7	3.06 ± 2.24	79	1	1.27 ± 2.49	308	8	2.60 ± 1.78
Children > 5 y.	98	1	1.02 ± 2.01	52	1	1.92 ± 2.77	150	2	1.33 ± 1.84
Children total	327	8	2.45 ± 1.68	131	2	1.53 ± 2.11	458	10	2.18 ± 1.34

Numerous factors defining the severity of the clinical manifestation (age, number of stools daily, general condition, signs of dehydration, laboratory results, etc.) as well as the subsequent necessity for hospitalization were analyzed, and all cases with confirmed cryptosporidiosis were stratified into four specific clinical forms (Figure 1) - acute diarrhoea with a risk of dehydration that requires hospitalization (n=3), mild diarrhoea with ambulatory treatment (n=4), persistent diarrhoea (n=1) and asymptomatic invasion (n=2). Thorough information of the clinical presentation, physical signs, general laboratory and parasitology results and the treatment are presented on Table 2 with the detailed descriptions of four of our clinical cases, each for every one of the defined clinical forms. No atypical or extraintestinal forms were detected in the examined children with immunosuppression caused by malnourishment or oncohematological disorders.

Fig. 1. Clinical forms of cryptosporidiosis in children

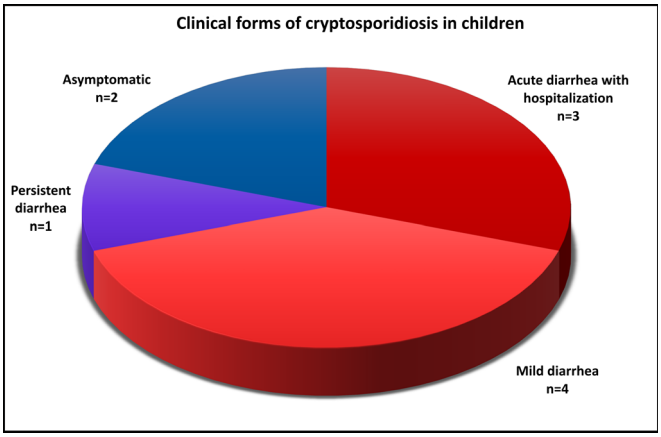

Table 2. Clinical features of four clinical cases with different clinical forms of cryptosporidiosis

Clinical form	**Acute diarrhearequiring hospitalization**	**Mild diarrheawith ambulatory treatment**	**Persistent diarrhea**	**Asymptomatic cryptosporidiosis**
Cases: **Age and sex:** **Residency:**	**Patient 1.** 2 years old / boy Small village / Varna district	**Patient 2.** 4 years old / boy Small village / Varna district	**Patient 3.** 6 years old / girl Small town / Northeastern Bulgaria	**Patient 4.** 5 years old / girl Small village / Northeastern Bulgaria.
Background/ Epidemiology history:	Frequent contacts with pets and other animals.	Frequent use of local water source (well) for drinking and household purposes.	Consumption of homegrown vegetables; contact with animals.	Contact with newborn pet (dog) and other animals (chickens, lambs).
Clinical presentation:	Acute onset with nausea, vomiting, stomach aches, watery diarrhea (> 5 stools/day) and fever (38^{o}C) for 3 days before hospitalization.	Acute onset with nausea, stomach aches, watery diarrhea for 2 days (> 5 stools/day) and before outpatient examination.	Acute diarrheal episode for 2 days (> 4 stools/day) followed by intermittentstomach aches and/or loose stools 3 weeks of before examination.	No diarrhea. *The stool sample is submitted for the required routine annual checkup for intestinal parasites of children between 2-7 years of age.*
Physical examination:	Moderately impaired general condition. Febrile (37.8^{o}C), adequate but week. Skin: pale and dry, with reduced turgor, without exanthema. Tongue: dry. Heart: tachycardia Lungs, liver, urinary and CNS: without significant changes.Abdomen: enhanced peristalsis and tenderness.	Mildly impaired general condition. Afebrile, adequate, irritable. Skin: without exanthema. Tongue: dry. Heart, lungs, liver urinary and CNS: without significant changes. Abdomen: enhanced peristalsis and tenderness.	Good general condition. Afebrile, adequate. Skin, tongue, throat: without significant changes. Heart, lungs, liver urinary and CNS: without significant changes. Abdomen: mild tenderness.	Physical status: without significant changes. Afebrile, adequate. Heart, lungs, liver urinary and CNS: without significant changes. Abdomen: without tenderness.
Laboratory tests:	***CBC:*** HGB-126 g/l, HCT- 0.344, RBC-4.61x10^{9}/l, WBC-8.42x10^{12}/l, PLT-382 x10^{9}/l; CRP: 1.04 mg/l Acid-base balance: **moderate metabolic acidosis** on admission, corrected on the second day. Electrolytes: K-**3.1**-4.87 **(mild hypokaliemia)**, Na-140-139, Cl-106-109 mm/l. ***Urinalysis:*****ketones – / +/positive** on the first day; without changes of the other parameters.	***CBC:*** HGB-139 g/l, HCT- 0.390, RBC-4.61x10^{9}/l, WBC-10.97x10^{12}/l, PLT-352 x10^{9}/l; CRP: 2.13 mg/l ***Urinalysis:*** without significant changes.	***CBC***: without significant changes. ***Urinalysis:*** without significant changes.	***CBC:*** without significant changes. ***Urinalysis:*** without significant changes.
Parasitology tests:	***mZNS:***high number of *Cryptosporidium spp.* oocysts	***mZNS:****Cryptosporidium spp.* oocysts. **ELISA:** /+/positive for *Cryptosporidium* spp. coproantigens	***mZNS:***small number of *Cryptosporidium spp.* oocysts. **Immunochromatographic tests:** /+/ copro-	***mZNS:***small number of *Cryptosporidium spp.* oocysts. **Immunochromatographic tests:** /+/ posi-

	Test for other IP: no cysts or ova detected	***Test for other IP:*** no cysts or ova detected	antigens for *Cryptosporidium spp.* and *G. duodenalis* ***Lugol'swet mount–*** /+/ positive for *G. duodenalis*cysts and *B. hominis*	tive for *Cryptosporidium spp.* coproantigens ***Test for other IP:*** no cysts or ova detected
Control tests:	***mZNS:*** /-/ negative on 20 day and 2 month	***mZNS:*** /-/ negative on 20 day and 2 month	***mZNS:*** /-/negative on 20 day and 2 month	***mZNS:***/-/negative on 10, 20 day and 2 month
Treatment:	**Ser. and Sol. glucose 5%** x 500 ml/dailyfor 5 days **Na bicarbonate** 8,4%, 10ml and **KCl**-15%/10 mlfor 1 day **Azithromycin**- 200 mg/ 5 ml powder for oral susp. - 2,25 ml (90mg)/ daily for 5 days **Enterol**–pulv. 250 mg, 2x1 sach./daily for 1 week and x1 for 3more weeks	**Oral rehydration therapy -Hydratin alpha** 4.1 g powder for oral susp. 5x1 daily in 200 ml water for 5 days. **Azithromycin** - 200 mg/ 5 ml powder for oral susp. - 2,25 ml (90mg)/ daily for5 days **Enterol**–pulv. 250 mg, 2x1 sach./daily for 1 week and x1 for 3more weeks	**Rehydration - not necessary** **Azithromycin-** 200 mg/ 5 ml powder for oral susp. - 2,25 ml (90mg)/ daily for 5 days; **Tinidazole**-40 mg/kg once(for treatment of *G. duodenalis*) **Enterol**–pulv. 250 mg, 2x1 sach./daily for 1 week and x1 for 3 weeks more.	**Rehydration - not necessary** **Etiological treatment-not necessary** **Enterol**–pulv. 250 mg, 1x1 sach./daily for 1 month

DISCUSSION

This is the first investigation of the distribution of cryptosporidiosis amongst children from the general population in Bulgaria.The results show that the estimated overall prevalence of the infection (2,16%) is comparable or even higher than the prevalence of other more well-known intestinal parasites as *B. hominis*- 2,29% [10], *E. vermicularis*-1,04% [11], *G. duodenalis* 0,70% [12] (studied in this age group in the similar time frame and region).For comparison, the overall prevalence of cryptosporidiosis the adults tested in the same period is only 0.70% ± 0.97% which indicates that the risk of contracting cryptosporidiosis in children is approximately 3 times higher than in adults (Odds Ratio = 3,122). Our proactive research in the risk groups probably is the reason why the result is significantly higher than the official data about the overall prevalence of cryptosporidiosis (0.36%) reported by the National Center of Infectious and Parasitic Diseases for a previous period [13].

Our findings show that the group of children 0-5 years old (prevalence of 2.6%) is at the highest risk of contracting the disease while in the children over 5 years the occurrence of cryptosporidiosis is almost halved (prevalence – 1.33%). This data agrees with the data for Europe[3, 5, 7] and the rest of the world[4,6]. The highest prevalence (3.06%) is found in the focus group of children 0-5 years with diarrhoea. This observation corresponds to the results reported by similar studies in other European countries - 3,2% in children with diarrhoea in Romania[5], 3% of children with diarrhoea in Netherlands with estimates of overall prevalence of2,91% in patients with diarrhoea for the Scandinavian countries [7], 2% in patients with diarrhoea in France, 1,65% in children with diarrhoea in Italy, and others [3, 4, 14].

In the scope of our investigation, we discovered that children of Northeastern Bulgaria might present with all possible clinical variants of the involvement of the gastrointestinal tract with *Cryptosporidium* spp. (figure 1, table. 2). In 1/3 of the cases, mainly in the youngest children (all were less than three years of age), the diarrhoea was so severe that it required hospitalization. The progress of the infection may include physical and laboratory signs of dehydration - pale and dry skin, tachycardia, impaired general condition, metabolic acidosis, hypokalemia, etc., as demonstrated by the first clinical case (table 2).

Another important presentation of cryptosporidiosis is the persistent (>two weeks) and in some cases chronic diarrhoea, especially in children with predispositions. In our case (Patient 3), we can attribute the prolonged infection to the comorbidity with other intestinal parasites (*B. hominis* and *G. duodenalis*).This shows that one positive result in just one field of investigation doesn't exclude the presence of other intestinal pathogens, thus requiring more specialized procedures as such for *Cryptosporidium* identification or multidisciplinary approach with microbiology and virology tests. Comorbidity of the GI tract with intestinal bacteria, viruses and parasites especially in the youngest children (<5 years) is a significant problem, demonstrated by numerous studies in the region [11,15–18].

Most common among the investigated children were the cases with mild diarrhoea (3-5 stools daily) that does not require hospitalization (n=4).Alongside with the asymptomatic infections, those clinical forms comprise 60% of the confirmed cases.At first glance, both conditions that lack significant physical and laboratory changes (patient 2 and patient 4) may seem clinically less relevant, but their epidemiological impact is very important. In the absence of our active research, those cases would have remained undiagnosed (as they usually do) and can persist as constant carriers of infec-

tious *Cryptosporidium* oocyst for a prolonged period, in some cases even over 2 months [1]. Furthermore, some studies demonstrate that even an asymptomatic infection can sometimes lead to stunt weight and growth in children [2].

CONCLUSIONS

This first epidemiological study and revealed cases of cryptosporidiosis in children of Northeastern Bulgaria demonstrate several important problems.

All possible clinical forms of cryptosporidiosis (from asymptomatic infection to the acute, severe diarrhoea with dehydration) occur in the population. This contradicts with the general approach in the clinical practice in the country where, in contrast with bacterial and viral agents, parasites are rarely considered by physicians as a potential cause of diarrhoeal disease.This tendency to underestimate cryptosporidiosis is especially hazardous in both risk groups of children and immunocompetent adults. Thus, it is necessary the examination for *cryptosporidium* infection to be mandatory in any type of diarrhoeal episode in the following risk groups:

- Children between 0-5 years with acute, persistent or chronic diarrhoea, particularly if it requires hospitalization.
- Children of all age groups (especially between 0-5 years) living in risk - poverty, malnutrition and growth retardation, minorities or immigrant populations, institutionalized etc.
- Children of all age groups with immunodeficiencies - primary immune deficiencies syndromes, acquired immune deficiency (HIV/AIDS), oncological diseases (lymphomas, leukemias and other oncohaematological disorders), bone marrow or solid organ transplant recipients, on hemodialysis etc.

Cryptosporidiosis is a disease that requires mandatory reporting from all European countries. However, in both the official information and systematic regional overviews the data conveyed from the Eastern European countries, including Bulgaria, comprise of only single accidentally discovered cases. Our proactive research demonstrates that cryptosporidiosis occurrence in children is much higher than previously reported. This translates erroneously into the lowest morbidity as compared to other parts of the continent and undoubtedly underestimates the burden of the infection in children.

Abbreviations (in text and tables):
IP - intestinal parasites
mZNS - modified Ziehl–Neelsen stain

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