Study on Vitamin D and nutritional status in children and adolescents with helminthiases in central Moldova

A. Voloc

Department of Pediatrics, Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

Corresponding author: voloc@mail.ru. Received November 05, 2015; accepted December 07, 2015

Abstract

Background: Vitamin D deficiency has a high prevalence among schoolchildren worldwide. The effects of deficiency include impaired immune response and increased risk of varios infections whose record is poor. Currently, scientists have the opinion that vitamin D status can be rightly considered as an indicator of health in a given country as well as a socio-economic indicator of each country. Unfortunately, very few researches are devoted to the role of vitamin D in parasitic invasions in children and associations between them, to be able to compare the resultants of our research with similar results in other countries.

Material and methods: The study included two groups: group 1 (control) comprised 34 girls and 37 boys from Chisinau, group 2 (study) included 97 girls and 129 boys from Orhei. The children in both groups were examined physically and anthropometrically. Vitamin D status was determined and copro-parasitological tests were carried out. There were determined hemoglobin levels and some biochemical markers in relation to their nutritional status.

Results: It was determined a poor vitamin D status in both cohorts and a high level of infestation with helminths without establishing any correlation between vitamin D status and the level of infestation. The prevalence of helminthiases varies depending on the type of helminth detected in each cohort. Intestinal parasitosis does not influence body weight, waist and body mass index.

Seven children with poly-invasion had a lower height, but their number was too small to validate the data. Children with parasitosis, regardless of its type, had some digestive signs more frequently than children without infestation and the incidence of pulmonary, hepatobiliary and dental diseases, as well as bone deformity was not influenced by the presence or absence of intestinal parasitosis.

Conclusions: Intestinal parasitoses do not influence body weight, waist and body mass index. Children with parasitosis, regardless of its type, have some digestive signs more frequently than children without infestation and the incidence of pulmonary, hepatobiliary and dental diseases as well as bone deformities was not influenced by the presence or absence of intestinal parasitosis. The children and adolescents from the boarding school in Orhei had a poor nutritional status due to an unbalanced diet with a low intake of milk/dairy products and meat and high in fiber.

Key words: Vitamin D status, helminth infestation, nutritional status.

Introduction

In the buletin of the World Health Organisation Experts Committee [1, 2] it is stated that intestinal parasitoses are prevalent worldwide, with a very high prevalence in some regions of the world. It was estimated, for example, that the global prevalence of soil-borne nematodes constitutes 100 million cases of Ascaris lumbricoides, 900 million cases of Ankylostome and 500 million cases of Trichuris trichiura. In some countries, the average global prevalence for Ascariasis may vary from 16 to 48% and in some places it may exceed 70%. Ascaris lumbricoides infestation is more prevalent in children under 17 years than in adults over 18 years [3]. In a questionnaire-based study conducted in Malaysia (Crompton D.W.T., 1985), on a representative sample of 25,000 children and adults, their age ranging from the neonatal age and up to 60 years, it was estimated that the global prevalence of intestinal parasitosis increases up to 39.6%, reaching 89% in children aged between 6 and 12 years.

In the last five years a series of publications have appeared which are entirely dedicated to the study of Giardia intestinalis infestation [4-15], which describe the role of giardiasis in the immune process [16], as well as the carcinogenic role of the pancreas [17, 18, 19, 20], the pathophysiological mechanism of giardiasis [21], giardiasis association with a diet poor in proteins [22], infestation influence on the nervous system of the host organism [23] and its prevalence in the world [24]. Publications certify the incidence of infestations in some European countries, the prevalence of giardiasis reaches 4.0% in Belgium [25], 1.5% in Germany [26], 0.4% - 6.2% in Italy [27, 28], 3.7% in Portugal [29], 5.4% in Spain [30], 1.3% in the UK [31], 1.4% in the US [32], 1.1 - 6.6% in Saudi Arabia [33]. In South Korea it is 2.5% [34], in Australia it ranges from 1.6% to 7.6% [35, 36] and in some regions in New Zealand it reaches 7.6 % [37].

Purpose

The aim of the study was to assess vitamin D status in children and adolescents in the boarding school of Orhei and those hospitalized in the public medical-sanitary institution, Municipal Clinical Children Hospital No. 1 in Chisinau, establishing its relationship with the level of helminth invasion, along with the evaluation of biochemical markers and calcium metabolism, comparing the prevalence in both cohorts.

Material and methods

The study included 2 lots: Lot 1: 226 children and adolescents, of whom 97 (43%) girls and 129 (57%) boys aged 7-16 years, observed at a regular medical and biological examination during the winter-spring months (from January to April) during the study process of schoolchildren at the boarding school in Orhei (latitude, 47 ° N). The children permanently lived in the boarding school during the study year, while in the summer they lived in their families in the rural area. There were excluded from the study only children with known chronic diseases and/or anthropometry data off the limits +/- 2 SD; Lot 2: 71 children of whom 33 (48.0%) girls and 38 (52.0%)

boys adjusted by age, being subjected to an identical clinical and biochemical examination while they were hospitalized for acute respiratory diseases in the same period in the Municipal Clinical Children Hospital No 1 (MCCH No 1) in Chisinau (latitude 47°21'N). These children lived in families in urban areas and did not have any endocrine, renal or neurological diseases, or any other known chronic diseases.

The presence of intestinal parasitoses, especially Enterobius vermicularis, Giardia lamblia, Ascaris lumbricoides and Trichuris trichiura, was determined by standard coprologic methods. Serum 25 (OH)D levels were determined using the international external scheme of quality assessment for vitamin D metabolite (DEQAS) in order to estimate the optimal levels of 25 (OH)D for all subjects included in the current cohort study.

The biochemical analyses included determination of the levels of serum calcium, phosphorus, magnesium, blood glucose, total protein, thymol probe, aminotransferases, urea and creatinine in the clinical-biochemical laboratory of MCCH No 1.

Given the objectives of the study, the children in both groups were investigated for helminths in the parasitological laboratory of the National Scientific and Practical Centre of Preventive Medicine. For this purpose, faeces and perianal scraping were used as biological material, applying coproparasitological methods, simple macroscopic examination, as well as dark-field examination of faeces or examination with a magnifying glass and microscopic method.

Statistical analysis. The level of serum 25 (OH) D was measured in serum samples stored at -20° C in Saint Vincent de Paul Hospital (Paris, France), using chromatographic assays of competitive protein binding with continuous DEQAS external quality assessment of 25 (OH) D tests. The samples were analyzed together with 20 DEQAS controls (range: 9-79 nmol/l). The values constituted – 0.11 ± 0.72 SD units (mean ± SD) of all laboratory media for each control. The normally distributed variables are reported as mean ± SD. The t-student test was used to compare the continuous variables normally distributed in pairs. The significant differences between groups <30 subjects were verified, applying the non-parametric test and variance analysis (ANOVA). The prevalence between groups was compared using the Chi-square test. All the analyses were performed using the Statview 5.0 (Abacus Concepts Inc., Berkeley, CA, USA). The value of P <0.05 was considered statistically significant.

Results

Nutritional intake. The children from the boarding school in Orhei were nourished according to a special and different menu, including at weekends. They had a grain-based diet with less consumption of meat, milk and dairy products. The food intake according to one-week menus for children in the boarding school during the study period was assessed on the basis of the table CIQUAL of nutritional composition of foods (http://www.afssa.fr/TableCIQUAL).

The daily intakes of the children of the public medical-sanitary institution, MCCH No 1 were calculated depending on the daily menu prepared by the dietitian of the setting. The comparative information on the nutrition of children in both groups is shown in table 1.

Using standard coprologic methods the presence of intestinal parasitosis was determined, namely *Enterobius vermicularis, Giardia lamblia, Ascaris lumbricoides and Trichuris trichiura.*

Values are expressed as mean ± ES or as a percentage of subjects with clinical symptoms or intestinal parasitoses in the study and control groups. They were compared using the unpaired t-student test (mean values) or the Chi square test (incidence).

The biochemical laboratory parameters were analyzed at the MCCH No1. They included serum calcium, phosphates, magnesium, total protein, creatinine, hemoglobin, glucose, alkaline phosphatase and transaminase activity ALT/AST.

226 participants studied in the boarding school and 71 children in the age-matched control group had comparable anthropometric parameters at birth and before puberty (table 2). However, the adolescents of both sexes from the boarding school had a smaller waist compared to the participants in the control group and according to the WHO growth reference curves. The two cohorts had poor dental health and an increased prevalence of gastrointestinal symptoms, mostly multiple, nausea (17%) and isolated abdominal pain (20%) in some cases and isolatedly.

Table 1

The average daily nutrient intakes in the research and control groups

Assessed parameters	Research group (Orhei)	Control group (Chișinău)	t	P
	M ± SD	M ± SD		
Total proteins (children) on body weight per day (g/kg)	2,5±0,41	2,6±0,12	0.2326	>0.05
Total proteins (adolescents) on body weight per day (g/kg)	1,8±0,32	2,3±0,43	0.9259	>0.05
Animal proteins (g)	24.1±0.41	48,6±1.24	18.7023	<0.001
Energy (kcal)	2842.6±46.74	3162.8±52.43	4.5587	<0.001
Phosphates (mg)	0,921 ±0165	1,03±0,22		
Magnesium (mg)	255.9±2.45	273.8±1.12	6.6543	<0.001
Calcium per day (mg)	493.5±8.47	930.6±12.02	29.7347	<0.001
Inclusive of dairy products (mg)	69.4±7.66	550.3±14.39	29.5031	<0.001
Vitamin D (mg)	2 ±0,12	2,01±0,05		

Table 2

Clinical characteristics of the two cohorts (m±DS and prevalence)

Population	Boarding school of Orhei	PMSI MCCH No 1	P
N	226	71	
Age (years)	11,4±1,8	11,1± 2,4	>0.05
Prepubertal (%)	52.0±3.32	46.0±5.91	>0.05
Sex % boys	57.0	52.0	>0.05
Sex % girls	43.0	48	>0.05
Parameters at birth			
Term (weeks)	39,4±0,8	39,2±1,6	>0.05
Weight at birth (kg)	3,11±0,42	3,08± 0,56	>0.05
Waist (cm)	50,5±2,3	50,4±2,1	>0.05
Anthropometry			
BMI (z-score by WHO)	- 0,205±1,041	- 0,471±1,141	<0.001
-Children (n)	- 0,325±1,107 (118)	- 0,261±1,228 (33)	>0.05
-Adolescents (n)	- 0,440±0,898 (108)	- 0,682±1,023 (38)	>0.05
Waist (z-score by WHO)	- 0,721±1,054	- 0,024±1,051	<0,001
-Children	- 0,088±0,964	- 0,155±1,101	>0.05
-Adolescents	- 1,009±1,126	0,106±1,000	<0,001
X2	X2 =12.1 GL=6 p>0.05		
Intestinal parasitosis (%)	49.0	63.0	
Enterobius vermicularis	40.0	20.0	0,0017
Ascaris lumbricoides	4.0	14.0	0,0104
Trichuris trichiuria	2.0	14.0	<0,0001
Giarda lamblia	-	15.0	
Poly-infestation	3.0	-	
X2	X2 =18.6 GL=5 p<0.01		

Table 3

Biochemical profile (m±ES) of children in the boarding school in Orhei (study group) and Municipal Clinical Children Hospital No 1 (control group)

	study group, n=226		control group, n=71		P
Children	118	52.0±4.59	44	46.0±8.68	>0.05
Adolescents	108	48.0±4.81	37	54.0±8.09	>0.05
Protein (g/l)	69.0±11.0		69.0±8.0		
Hb (g/l)	11,2±1,1		11,9±0,4		
Glucose (mmol/l)	4,2±0,8		4,3±0,7		<0,0001
Creatinine (µmol/l)					
children	41.0±12.0		54.0±10.0		
adolescents	58.0±13.0		60.0±11.0		<0,0001
Total calcium (mmol/l)	2,14±0,31		2,29±0, 23		0,0007
Corrected Ca (mmol/l)*	2,22±0,31		2,36±0,24		0,0025
Phosphate (mmol/l)	1,36±0,38		1,22±0,23		0,0072
Magnesium (mmol/l)	0,70±0,14		0,83±0,18		<0,001
PA (UI/l)					
children	453.0±202.0		407.0±123.0		
adolescents	596.0±227.0		384.0±136.0		<0,001
25(OH)D (nmol/l)	44.0±16.0		36.0±12.0		<0,001
25(OH)D (ng/ml)	18.0±6.0		14.0±5.0		<0,001

* The values of total serum calcium were corrected for protidemia. Significant differences were determined in the boarding school group and control group (unpaired t-student test and U Mann-Whitney).

The parasitic infestations were common, with significant differences concerning the identified parasites (table 2). Compared with the age-matched control group, the participants from the boarding school had lower values of calcium, serum magnesium and hemoglobin, and a higher average level of serum phosphate (table 3). In addition, the children in the study group from the boarding school were recorded lower serum creatinine values before puberty and higher alkaline phosphatase activity during puberty compared to the age-matched control group (table 4).

Assessment of vitamin D status and its clinical impact

The circulating values of 25 (OH) D and, thus, vitamin D reserves have been rated as "satisfactory" values so far, if they were within the mean values ± 1 or ± 2 standard deviations in the adult population. Sun exposure influences the reserves of vitamin D, "normal" values of 25-hydroxyvitamin D, being different depending on the season - summer/autumn or winter/spring, the geographical location of the country and its policy of enriching foods with vitamin D.

Table 4

Clinical characteristics in the cohort of Orhei boarding school according to the level of 25 (OH) D (m ± SD)

25-(OH)D	≤30 nmol/l	31-40 nmol/l	41-50 nmol/l	51-≥75 nmol/l
N 25-(OH)D (nmol/l) 25-(OH)D (ng/ml)	47 26,5±3,5 10,6±1,4	67 35,6±2,8 14,2±1,1	53 45,9±2,7 18,4±1,1	59 65,2±12,0 26,0±4,8
Age (years) -Children (n) -Adolescents (n)	11,8±1,9 9,9±1,1 (20) 13,2±1,0 (27)	11,5±1,6 10,2±0,9 (33) 12,7±1,0 (34)	11,3±2,0 9,7±1,2 (29) 13,1±1,1 (24)	11,2±1,7 10,2±1,1 (36) 12,9±0,9 (23)
BMI (z-score): -Children -Adolescents	 - 0,10±0,83 - 0,18±1,04	 -0,24±1,00 -0,44±1,30	 - 0,07±1,08 - 0,083±0,95	 0,07±0,88 -0,64±1,05
Height (z-score): -Children -Adolescents	 - 0,20±0,91 - 0,74±1,10	 - 0,62±0,86 - 1,04±1,04	 - 0,50±0,97 - 0,92±1,46	 - 0,42±0,87 -1,36±0,88
Prevalence: Digestive disorders Parasitoses	 71% 55%	 64% 43%	 68% 53%	 46% 47%

Implementation of a tailored prophylaxis has helped significantly reduce the incidence of severe vitamin D deficiency in all the Western countries, except the elderly, the clinical trials being focused on the effects of long-term moderate deficit.

Over time, scientists have gradually replaced the concept of "normal values" of 25 (OH) D with the term "required values" and with the notion of "threshold" below which some medium and long-term pathological changes may occur in apparently healthy individuals. The clinical consequences of this limit are taken into account when defining the value of this level, however, to date, there is no consensus on this level. However, from a practical perspective, it is useful to note: a) the level of "deficiency" below which the short-term pathological deficiency risk is significant and requires immediate correction. The multiple dosage of 25 (OH) D performed in neonates, infants and children with clinical signs of deficiency rickets (skeletal deformities and / or neurological signs as a result of hypocalcaemia) demonstrates eloquently the close correlation of these signs with values under 10 to 12 ng/ml (25-30 nnmol/l) of 25 (OH) D; b) the level of "insufficiency " of vitamin D status under which there is a risk of developing some long-term changes and may involve specific vitamin D prevention. The "insufficiency" threshold values vary in different authors from 20 to 52 ng/ml (50 - 130 nmol/l).

The children and adolescents examined in the rural boarding school in the period between January and April had a mean value ± SD of serum 25 (OH) D of 44 ± 16 nmol/l, with a prevalence of values of 25 (OH) D ≤ 30 ≤ 50 ≤ 75 nmol / l respectively equal to 21, 53 and 26%. The status of vitamin D was higher than the status measured in the children and adolescents in the control group who lived in Chisinau, either in flat (35.6 ± 1.8 nmol / l) or house (37.0 ± 2 3 nmol / l). It was not influenced by the pubertal maturation, but it varied by gender: girls from the boarding school had average values of 25 (OH) D less than boys (40.2 ± 14.8 versus 46.5 ± 16.0 nmol / l, p = 0.0031).

There are marked differences compared to the prevalence values observed in the case of 25 (OH) D ≤30 nmol/l. The statistical Chi-square test for prevalence and unpaired t-Student test were applied, as well as U Mann-Whitney test for age and anthropometry was used. The subgroups of sex and pubertal maturation were too small (less than 30 people) for the Chi-square analysis of prevalence.

Finally, the serum levels of 25 (OH) D were positively associated with serum calcium levels (r = 0.202, P = 0.0024)

Table 5

Biochemical indices depending on 25 (OH) D level in Orhei boarding school (m ± SD)

25-(OH)D (nmol/l)	≤30 nmol/l (n=47)	31-40 nmol/l (n=67)	41-50 nmol/l (n=53)	51-≥75 nmol/l (n=59)
Protein (g/l)	70±10	67±10	71±10	69±12
Hb (g/l)	11,0±1,1	11,4±1,0	11,4±1,0	11,2±1,1
Glucose (mmol/l)	4,2±0,8	4,0±0,8	4,2±0,8	4,2±0,8
Creatinine (µmol/l) -Children (n) -Adolescents (n)	 44±14 (20) 60±14 (27)	 40±12 (33) 56±13 (34)	 41±12 (29) 59±12 (24)	 41±9 (36) 61±14 (23)
Total Ca (mmol/l)	2,10±0,26 0,0205	2,12±0,30 0,0340	2,11±0,33 0,0259	2,24±0,32
Corrected Ca (mmol/l)	2,15±0,28 0,0083	2,22±0,31	2,16±0,35 0,0100	2,32±0,35
Phosphates (mmol/l)	1,33±0,34	1,35±0,41	1,37±0,38	1,39±0,37
Magnesium (mmol/l)	0,70±0,14	0,67±0,12	0,71±0,14	0,72±0,15
PA (UI/l) -Children -Adolescents	 534±1480,0138 596±228	 439±231 593±219	 432±187 633±244	 437±211 573±228

Table 6

Frequency of parasitoses in both groups

Type of parasitosis	<30				30-50				51 >			
	Chișinău n=37	Orhei n=46	T	P	Chișinău n=25	Orhei n=112	T	p	Chișinău n=9	Orhei n=68	T	p
	P±ES, %	P±ES, %			P±ES, %	P±ES, %			P±ES, %	P±ES, %		
1. Ascariasis	10,0±2,91	43,0±3,25	7,5	••••	6,0±2,31	5,0±1,43	0,4	•	6,0±2,31	3,0±1,12	1,2	•
2. Enterobiasis	24,0±4,15	44,0±3,26	3,8	••••	43,0±4,81	38,0±3,19	0,9	•	29,0±4,41	40,0±3,22	2,0	••
3. Giardiasis	15,0±3,47	-	4,3	••••	-	-			18,0±3,73	-	4,8	••••
4. Trichuriasis	12,0±3,16	4,0±1,29	2,4	••	1,0±0,97	1,0±0,65	0	•	12,0±3,16	1,0±0,65	3,4	••••

• p>0,05; •• p<0,05; •••• p<0,001

Table 7

Vitamin D status and calcium metabolism in relation to intestinal parasitoses

Parazitosis	Age, years	Corrected Calcium, mmol/l	Phosphates, mmol/l	Magnesium, mmol/l	25(OH)D, ng/ml
Absent (N=161)	10,8±2,9	2,25±0,31	1,33±0,34	0,74±0,16	18,5±6,3 (86)
Enterobiasis (N=114)	10,6±2,4	2,21±0,35	1,32±0,38	0,71±0,15	18,0±5,3 (70)
Ascariasis (N=19)	11,1±2,2	2,39±0,25	1,37±0,25	0,71±0,12	16,1±6,5 (9)
Trichuriasis (N=18)	11,1±3,3	2,31±0,27	1,26±0,20	0,75±0,16	15,1±4,2 (13)
Giardiasis (N=13)	10,4±4,0	2,51±0,15 (0,007)	1,32±0,18	0,94±0,23 (p<0,0001)	15,0±8,0 (6)
Poly-invasion (N=7)	11,5±2,0	2,23±0,32	1,34±0,49	0,77±0,11	18,0±4,7 (4)

Values represent the mean ± SD. Determination of the 25 (OH) D level was not possible in all children. The number of samples in which 25 (OH) D was determined is noted in parentheses. The significantly different values observed in uninfected children are noted by indicating the p value.

Table 8

Anthropometric and biochemical data depending on the infestation with intestinal parasites

	Weight (m±DS)	Waist (m±DS)	BMI (kg/m2)	Protein	Glucose	Creatinine	Hb
Absence of parasitosis (N=161)	-0,12±1,16	-0,12±1,34	16,9±2,4	69,2±9,5	4,17±0,87	54,2±14,2	118,2±9,0
Enterobiasis (N=114)	-0,09±1,13	-0,30±1,28	17,1±2,2	69,2±11,3	4,33±0,79	46,1±13,1 (p<0,0001)	111,8±9,1 (p<0,0001)
Ascariasis (N=19)	0,00±1,24	0,26±1,35	17,2±2,8	66,4±8,6	4,32±0,66	54,2±15,5	108,6±10,8 (p<0,0001)
Trichuriasis (N=18)	-0,09±1,32	0,54±1,09 (p=0,04)	16,6±2,6	69,6±8,4	4,04±0,85	56,5±11,6	113,6±13,6 (p=0,047)
Giardiasis (N=13)	-0,46±1,27	0,18±1,22	16,7±2,6	72,3±6,8	4,28±0,67	61,1±12,3	117,5±2,5
Poly-invasion (N=7)	-0,21±1,44	-1,18±1,17 (p=0,036)	18,1±2,1	68,4±10,2	4,29±0,77	47,1±8,6	105,1±7,7 (p=0,0003)

The mean values ± SD. Values significantly different from those found in uninfected children are **noted** (ANOVA).

in the study group: in participants with values of 25 (OH) D ≤ 30 nmol / l and even in those with intermediate values (30-50 nmol / l) serum calcium level was lower than in those with 25 (OH) D> 51 nmol / l (table 5).

On the contrary, serum calcium was not associated with the level of 25 (OH) D in the control cohort and the participants in the study group had significantly lower serum calcium levels than those in the control group when their level of 25 (OH) D was ≤ 30 nmol / l (2.15 ± 0.28 versus 2.41 ± 0.16 mmol / l, P <0.0001), but not in the case when the values of 25 (OH) D were greater than 51 nmol / l (2.32 ± 0.35 versus 2.50 ± 0.12 mmol / l).

The observed values are significantly different from those observed in the case of 25 (OH) D values greater than 50 nmol/l (using paired and unpaired t-student test and U Mann-Whitney test).

The lack of association with serum aminotransferase activity suggests that high alkaline phosphatase activity detected in children in the study group during puberty, reflects an increased turnover of bone tissue, possibly due to calcium deficiency.

Finally, 25 (OH) D level was positively associated with serum calcium level, with optimum 25 (OH) D values greater than 50 nmol/l. There was established no association of vitamin D level with clinical calcium-dependent symptoms, especially with hypocalcemia, probably because the participants in the study group were older and / or because their level of hypocalcemia and vitamin D deficit was moderate compared to previously reported cases in the literature [38, 39].

The status of vitamin D varies with the season in countries with temperate climate, being lower during the winter-spring period, when the ultraviolet solar spectrum energy is insufficient to produce vitamin D in the skin. But, a diet based on grains with restricted access to meat products, milk and dairy products had probably a major importance, an unusual diet for children in the Republic of Moldova, including those in the control group in urban areas, who theoretically receive an adequate intake of calcium / milk.

Helminthiases, vitamin D status, anthropometric and biochemical parameters and clinical manifestations

Comparing the frequency of helminthiases in both cohorts, we found an equivalent frequency (60% in Chisinau and 52% in Orhei). At the same time, their frequency varies depending on the parasite found (table 6).

Thus,the frequency of ascariasis, trichuriasis and giardiasis was higher in Chisinau than in Orhei, and, conversely, the frequency of enterobiasis was lower in Chisinau than in Orhei. We found no association between 25 (OH) D level and the presence or absence of intestinal parasitosis or its type.

Performing an ample analysis of the possible association between infestation with intestinal parasites and vitamin D status and the level of calcium, serum magnesium and alkaline phosphatase activity, we have observed a significant difference in some biochemical indices (table 7, 8).

Intestinal parasitoses do not influence body weight, waist and body mass index (BMI). Seven children with poly-invasion had a lower height, but their number was too small to validate the data. In contrast, lower concentrations of serum hemoglobin were detected in children with all types of parasitoses.

The other biochemical parameters did not show any difference in infected or uninfected children, with some exceptions:

- lower serum creatinine values were recorded in children with enterobiasis;
- higher values of serum calcium and magnesium were recorded in 13 children infected with giardia.

These findings remain unexplained and would require further study for confirmation.

Children with parasitosis, regardless of its type, had some digestive signs more frequently than children without infestation (table 9). However, the frequency of pulmonary, hepatobiliary and dental diseases as well as bone deformities was not influenced by the presence or absence of intestinal parasitoses.

Discussion

Despite the tense parasitic epidemiological status in all countries of the world and the large number of researches on studying just parasites alone, few researches are devoted to the study of vitamin D status and its correlation with intestinal parasitoses. The study to which we had access [40], conducted in a rural school in Mexico on a sample of 284 children (8 ± 1.6 years) and which researched the impact of Ascaris lumbricoides and Entamoeba coli invasion on the micronutritional status through rapid diagnostic methods, determined that 20% of the students involved in the study were infected with Echerichia coli and 16% with Ascaris lumbricoides. The prevalence of vitamin D deficiency was 28% and zinc deficiency constituted 18%. Perhaps the poor sanitary and hygienic conditions in which the children from metropolis lived in made researches detect such a prevalence. In one relatively recent publication [41] it is stated that vitamin D deficiency has a high prevalence among students worldwide. The deficiency effects include alterations in the immune response and an increased risk to various infections, the records of which are

Table 9

Prevalence of digestive signs and other clinical signs depending on the presence of intestinal parasitoses

	Absent	Enterobiasis	Ascariasis	Trichuriasis	Giardiasis	Poly-invasion
N / Digestive signs	172	118	21	20	14	7
Nausea	21 (12%)	10 (8%)	0	4	0	0
Pain	24 (14%)	9 (8%)	1	1	0	1
Some signs	25 (14%)	89 (76%)	18	14	14	6
Hepatic	8 (5%)	7 (6%)	2	0	0	0
Respiratory	56 (32%)	29 (24%)	12	16	14	0
Dental alterations	125 (73%)	103 (87%)	18	18	14	7
Bone deformations	67 (39%)	53 (45%)	14	9	4	4

Values represent the number of children. In addition, the percentage of children with any clinical signs considered were calculated in populations of more than 100 people (infected and uninfected children with enterobiasis).

low [1]. According to some ample studies made in Panama, it was pointed out a low digestion of lactose and intolerance to carbohydrates in preschool age children infected with Ascaris lumbricoides, which led, in turn, to accelerated intestinal transit [42] and probably a lower absorption of vitamin D and calcium in the diet, which in our view, has worsened the uptake of micronutrients in children of both groups involved in our study compromising the bioavailability of calcium from the diet low in milk/dairy products and rich in fiber mainly in children from the boarding school in Orhei.

Some authors (Issenman 1987) have mentioned that in case of giardiasis, parasites compete with enterocytes, causing malabsorption, including vitamin D malabsorption. It might be assumed in our study that one of the causes of anemia in children of both groups may be a deficiency of vitamin D. Some authors hypothesize that iron deficiency causes malabsorption of fats and vitamin D as a fat soluble molecule, such as in the case of giardiasis, or vice versa, induction of anemia as a result of intense inflammatory processes in the gut and bone marrow myelofibrosis associated with vitamin D deficiency [43, 44] The obtained results require an analytical confrontation with the results reported in different parts of the world, to try to find an explanation for a very high proportion of anemia, deficiency of micronutrients, including deficiency of vitamin D.

The literature reports that in case of giardiasis, the malabsorption syndrome is present in 90% of cases in children and 30% in adults; the malabsorption in this population being characterized by weight loss and biological malabsorption, it being most commonly partial, especially fats malabsorption (steatorrhea), carbohydrates (D-xylose), folic acid and vitamins A and B12, accompanied by villous (total, partial or subtotal) atrophy and megaloblastic anemia by faulty absorption of folic acid and vitamin B12 [45].

It might be assumed that infestation with Trichuris trichiura, which lives mainly at the colon level and, most frequently, without any clinical manifestations, might, along with other causative factors, be at the origin of anemia in children and adolescents in both groups. Therefore, anemia in these subjects can be accounted for the fact that the adult parasite penetrates the intestinal mucosa causing an inflammation with lymphoplasmacytic and eosinophilic infiltration. It was estimated that a worm can consume about 0,005 ml of blood a day, while a helminth invading a person, can amount on average over 1,000 worms [46]. Iron deficiency is the most common cause of anemia, but the deficiency in other micronutrients is also involved (folic acid, vitamin B12, vitamin A). Regardless of acute or chronic inflammation, intestinal parasitoses are equally involved in the development of anemia [47].

The prevalence of anemia in our study was 100%. This value is higher than the 40% threshold set by WHO for a severe endemic anemia in a population group [48]. In fact, it does not reflect the prevalence of anemia among the general population and accounts for the study carried out on children in a hospital and school environment where there are specific predisposing factors (acute respiratory infection in children hospitalized in MCCH No 1, basic hygiene and poor nutrition along with a high degree of helminth infestation both in Chisinau and Orhei).

Worldwide around the globe, children are very susceptible to Enterobius vermicularis infestation. However, it seems that the disease is more present in temperate regions and highly developed countries than in tropical and subtropical regions [49]. It was certified that Enterobis vermicularis is the only parasite that is most frequently encountered in developed countries. However, this worm is sensed by the population as a "shameful" disease, constituting a social stigma in prosperous and highly developed societies [2].

Conclusions

Our study has not found any close relationship between the level of vitamin D deficiency and helminth infestation and vice versa.

The study results show that Giardiasis, Ascariasis, Enterobiasis and Trichuriasis are the most common intestinal parasites in children and adolescents in central Moldova, at least in the subjects involved in the study of both settings. The study allowed us to conclude that intestinal parasitoses do not influence body weight, waist and BMI. Seven children with poly invasion had a lower height, but their number was too small to validate the data.

Children with parasitosis, regardless of its type, have some digestive signs more frequently than children without infestation and the incidence of pulmonary, hepatobiliary and dental diseases as well as bone deformities was not influenced by the presence or absence of intestinal parasitosis. The children and adolescents from the boarding school in Orhei had a poor nutritional status due to an unbalanced diet with a low intake of milk/dairy products and meat and high in fiber.

References

1. Thornton KA, Marhn C, Mora-Plazas M, et al. Vitamin D deficiency associated with increased incidence of gastrointestinal and ear infections in school-age children. Pediatr Infect Dis J. 2013;32(6):585-93.
2. Bunnag D, Cabrera BD, Ceompton DWT, et al. La réunion du Comité OMS d'experts sur la lutte contre les parasitoses intestinales. Génève, Suisse. 3 – 7 mars, 1986.
3. Warken KS, Mahomed AAF. Prevalence of Ascaris lumbricoides infestation. Tropical and geographical medecine. 1984;279-87.
4. Beatty J, Akierman S, Rioux K, et al. Gut microbiota biofilm disruptions by Giardia: pathology in human enterocytes and germ-free mice. Experimental Biology. Boston Convention & Exhibition Center April 20-24 2013. Meeting Abstracts, 21.
5. Ankarklev J, Jerlström-Hultqvist J, Ringqvist E, et al. Behind the smile: cell biology and disease mechanisms of Giardia species. Nat Rev Microbiol. 2010;8:413-422.
6. Buret AG, Bhargava A. Modulatory mechanisms of enterocyte apoptosis by viral, bacterial and parasitic pathogens. Crit Rev Microbiol. 2013. Epub ahead of print.
7. Stadelmann B, Merino MC, Persson L, et al. Arginine consumption by the intestinal parasite Giardia intestinalis reduces proliferation of intestinal epithelial cells. PLoS One. 2012;7.
8. Maia-Brigagão C, Morgado-Díaz JA, De Souza W. Giardia disrupts the arrangement of tight, adherent and desmosomal junction proteins of intestinal cells. Parasitol Int. 2012;61:280-287.

9. Ettehad GH, Daryani A, Nemati A. Effect of Giardia infection on nutritional status in primary schoolchildren, in northwest Iran. Pak J Biol Sci. 2010;13:229-234.
10. Sweeny JP, Ryan UM, Robertson ID, et al. Cryptosporidium and Giardia associated with reduced lamb carcase productivity. Vet Parasitol. 2011;182:127-139.
11. Cantey PT, Roy S, Lee B, et al. Study of nonoutbreak giardiasis: novel findings and implications for research. Am J Med. 2011;124:1175.
12. Veenemans J, Mank T, Ottenhof M, et al. Protection against diarrhea associated with Giardia intestinalis is lost with multi-nutrient supplementation: a study in Tanzanian children. PLoS Negl Trop Dis. 2011;5:1158.
13. Naess H, Nyland M, Hausken T, et al. Chronic fatigue syndrome after Giardia enteritis: clinical characteristics, disability and long-term sickness absence. BMC Gastroenterol. 2012;12:13.
14. Wood NJ. Infection: Giardia lamblia is associated with an increased risk of both IBS and chronic fatigue that persists for at least 3 years. Nat Rev Gastroenterol Hepatol. 2011;8:597.
15. Lander RL, Lander AG, Houghton L, et al. Factors influencing growth and intestinal parasitic infections in preschoolers attending philanthropic daycare centers in Salvador, Northeast Region of Brazil. Cad Saude Publica. 2012;28:2177-88.
16. Solaymani-Mohammadi S, Singer SM. Giardia duodenalis: the double-edged sword of immune responses in giardiasis. Exp Parasitol. 2010;126:292-297.
17. Kurita A, Maguchi H, Takahashi K, et al. Small pancreatic cancer with giardiasis: a case report. Pancreas. 2010;39:943-945.
18. Furukawa M, Lee L, Ikegami T, et al. Giardiasis in the pancreas accompanied by pancreatic cancer. Pancreas. 2011;40:168-169.
19. Mitchell CM, Bradford CM, Kapur U. Giardia lamblia trophozoites in an ultrasound-guided fine-needle aspiration of a pancreatic mucinous neoplasm. Diagn Cytopathol. 2011;39:352-353.
20. Nagasaki T, Komatsu H, Shibata Y, et al. A rare case of gallbladder cancer with giardiasis. Nihon Shokakibyo Gakkai Zasshi. 2011;108:275-279.
21. Cotton JA, Beatty JK, Buret AG. Host parasite interactions and pathophysiology in Giardia infections.Int J Parasitol. 2011;41:925-933.
22. Gomes MA, de Oliveira DR, de Freitas SE, et al. Effect of giardiasis combined with low-protein diet on intestinal absorption of glucose and electrolytes in gerbils. Exp Parasitol. 2012;131:448-451.
23. Hacker VL, Stark D, Thomas S. Validation of the stroke aphasic depression questionnaire using the brief assessment schedule depression cards in an acute stroke sample. Br J Clin Psychol. 2010;49:123-127.
24. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of Giardia species and giardiasis. Clin Microbiol Rev. 2011;24:110-140.
25. Geurden T B, Levecke S M, Caccio A, et al. Multilocus genotyping of Cryptosporidium and Giardia in non-outbreak related cases of diarrhoea in human patients in Belgium. Parasitology. 2009;136:1161-68.
26. Sagebiel D, Weitzel T, Stark K, et al. Giardiasis in kindergartens: prevalence study in Berlin. Germany.Parasitol. Res. 2006;105:681-687.
27. Crotti D, D'Annibale M L, Fonzo G, et al. Dientamoeba fragilis is more prevalent than Giardia duodenalis in children and adults attending a day care centre in Central Italy. Parasite. 2005;12:165-170.
28. Giangaspero A, Berrilli F, Brandonisio O. Giardia and Cryptosporidium and public health: the epidemiological scenario from the Italian perspective. Parasitol. Res. 2007;101:1169-82.
29. Almeida AA, Delgado ML, Soares SC, et al. Genotype analysis of Giardia isolated from asymptomatic children in northern Portugal. J. Eukaryot. Microbiol. 2006;53:177-178.
30. Manzardo C, Trevino B, Gomezi Prat J, et al. Communicable diseases in the immigrant population attended to in a tropical medicine unit: epidemiological aspects and public health issues. Travel Med. Infect. Dis. 2008;6:4-11.
31. Davies AP, Campbell B, Evans MR, et al. Asymptomatic carriage of protozoan parasites in children in day care centers in the United Kingdom. Pediatr. Infect. Dis. J. 2009;28:838-840.
32. Church C, Neill A, Schotthoefer A. Seasonality of parasitic gut infections in humans in the Rocky Mountain region, United States. J. Parasitol. 2010;96:104-106.
33. Al-Megrin W A. Intestinal parasites infection among immunocompromised patients in Riyadh, Saudi Arabia. Pak. J. Biol. Sci. 2010;13:390-394.
34. Huh JW, Moon SG, Lim YH. A survey of intestinal protozoan infections among gastroenteritis patients during a 3-year period (2004-2006) in Gyeonggido (province), South Korea. Korean J. Parasitol.2009;47:303-305.
35. Hellard ME, Sinclair MI, Hogg GG, et al. Prevalence of enteric pathogens among community based asymptomatic individuals. J. Gastroenterol. Hepatol. 2000;15:290-293.
36. Read C, Walters J, Robertson ID, et al. Correlation between genotype of Giardia duodenalis and diarrhoea. Int. J. Parasitol. 2002;32:229-231.
37. Learmonth JJ, Ionas G, Pita AB, et al. Identification and genetic characterisation of Giardia and Cryptosporidium strains in humans and dairy cattle in the Waikato Region of New Zealand. Water Sci. Technol. 2003;47:21-26.
38. Esterle L. Adverse interaction of low-calcium diet and low 25(OH)D levels on lumbar spine mineralization in late-pubertal girls. J Bone Miner Res. 2010;117-20.
39. Kardinaal AFM, Ando S, Charles P, et al. Dietary calcium and bone density in adolescent girls and young women in Europe. J Bone Miner Res. 1999;14:583-592.
40. Garcial O, del Carmen Caamaño M, Zavala G, et al. Infection with Ascaris lumbricoides or Entamoeba Coli Affect Differently Zinc Status in School-aged Mexican Children. The FASEB Jurnal. 2015;29:754-4.
41. Kenneth N, Opara Ph D, Nsima C, et al. The impact of Intestinal Parasitic Infections on the Nutritional Status of Rural and Urban School-Aged Children in Nigeria. International Jurnal of MCH and AIDS. 2012;1(1):73-82.
42. Keusch G T. The biology of parasitic infection. Workshop on International Nutrition and Parasitic Diseases. Reviews of infectious diseases. 1982;4:735-911.
43. Mehta S, Giovannucci E, Mugusi F M. Vitamin D status of HIV – incefted women and its association with HIV disease progression, anemia, and mortality. PLOS One. 2010;5(1).
44. Stephan RE, Gerhard RV, Spiegelman D, et al. Intestinal absorbtion and vitamin D levels – is a new focus needed? Digest. Dis. 2012;30:73-80.
45. Trabelsi S, Oueslati J, Aouinet A, et al. Les anémies d'origine parasitaire. La Tunisie médicale. 2014;92(6):361-367.
46. Bourée P. Trichocéphalose. In: Nozais JP, Datry A, Danis M,editors. Traité de parasitologie médicale. Paris: Pradel, 1987;157-63.
47. Kedy Koum D, Ngouadjeu Dongho Tsakeu E, Ngo Sack F. . [. Pan Afr Med J. 2013;16:91.
48. WHO. Worldwide prevalence of anaemia 1993-2005. WHO Global Database on Anaemia. Health Nutrition. Helen Keller International. Available at http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf. Access 1 September 2013.
49. Vanden Bossche H. Economic aspects of parasitic disease: Proceedings of an international workshop. Social Science and medecine. 1984;19:1013-26.

Engystol®

-Heel
Healthcare designed by nature

Properties

- Complex action: antiviral, immunomodulatory, anti-inflammatory and detoxification.
- Regeneration of SH-groups of enzymes at intoxications.
- Possibility of ARVI and influenza prevention.
- Favourable safety profile, good tolerability.
- Can be used during pregnancy, breastfeeding, in childhood (pills – from birth).
- Preservation effect during prolonged therapy of chronic viral infections.
- Scientifically and clinically proven effectiveness.
- It is combined with other medicines.

Indications

- Acute and chronic infectious diseases of viral origin.
- Influenza, ARVI, bronchitis, pneumonia, whooping cough.
- Viral hepatitis (acute and chronic).
- Herpes, cytomegalovirus infection.

Prevention and treatment of viral diseases

Prevention of viral diseases during the whole autumn-winter period		
Engystol	Pills	Injectable solution
	Single age dose (SAD) – 1 time/day (in the morning) and/or	SAD – intramuscularly (i/m), subcutaneously (s/c) 1 time per day.
Prevention of ARVI and influenza during the period of epidemics		
Engystol	Pills	Injectable solution
	SAD – 2 times per day (in the morning and in the evening) and/or	SAD – i/m, s/c 2 times per week.
General and acute treatment of ARVI		
Engystol	Pills	Injectable solution
	SAD – From the onset (acute): 1 tablet every 15-20 minutes for a maximum of 6 doses per day; after the onset of symptoms – 1 tablet 3 times daily.	SAD – From the onset (acute): one ampoule once daily, after the onset of symptoms – i/m, s/c 3 times per week.

Representation of Biologische Heilmittel Heel GmbH in the Republic of Moldova
65, Tighina Street, Office 309, Chisinau, the Republic of Moldova
www.heelmd.org. Telephone: +(373)22507780