Parasitological Examination among Local Residents in Afghanistan, Central African Republic and Kosovo Conducted by the Polish Military Health Service.*

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RESUME

Enquête parasitologique dans les populations d'Afghanistan, de République Centrafricaine et du Kosovo, menée par me service de santé de l'armée polonaise.

Objectifs: *De 2011 à 2015, les forces armées polonaises ont réalisé des missions en Asie, en Afrique et en Europe. Une partie de ces missions était l'aide humanitaire aux populations dans le domaine des maladies infectieuses. Le but de cette étude est de présenter les résultats d'analyses parasitologiques effectuées chez des habitants d'Afghanistan, de République centrafricaine et du Kosovo pour connaître la prévalence des parasitoses intestinales et pour définir des stratégies de déparasitage.*
Matériels et méthodes: *Cette étude a concerné trois types de populations: en Afghanistan (3 146 personnes, patients de l'hôpital provincial de Ghazni, lycéens de la province de Ghazni et soldats de l'armée nationale afghane), République Centrafricaine (3 209 personnes, habitants d'origine pygmée de Monassao et Bagandou, habitants de Bangui et Bagandou) et Kosovo (632 personnes; écoliers du primaire de la province de Kaçanik). Chaque individu a fourni de 1 à 3 échantillons de selles qui ont été fixés dans une solution de formol à 10 % et transportés en Pologne où ils ont été poolés et examinés par examen direct et après deux méthodes de concentration: flottation de Fülleborn et décantation en eau distillée.*
Résultats: *Une infestation parasitaire a été identifiée chez 1 225 des Afghans (prévalence de 38,9 %, les parasites les plus fréquents étant Ascaris lumbricoides 19.6 %, Giardia intestinalis 15.3 %), chez 2 231 habitants de la République Centrafricaine (prévalence de 69,5 %, les parasites les plus fréquents étant: Ancylostoma duodenale/Necator americanus 40.0 %, Ascaris lumbricoides 36.5 %), et 76 habitants du Kosovo (prévalence de 12 % avec une plus grande fréquence de Giardia intestinalis 10.0 %). Les sujets infectés ont reçu un traitement antiparasitaire selon les recommandations.*
Conclusions: *Ces résultats montrent des différences significatives entre les pays. La large variété des parasites en Afghanistan et la prédominance des protozoaires au Kosovo pourraient conduire à réévaluer les procédures de déparasitage recommandées par l'OMS (doses uniques de 400 mg d'albendazole ou de 500 mg de melbendazole). Avant d'entreprendre un déparasitage dans les pays en développement, il serait pertinent de faire une étude parasitologique permettant de définir la prévalence de l'infestation et de connaître les principales espèces en cause dans telle ou telle population.*

KEYWORDS: Intestinal parasites, Light microscopy, Afghanistan, CAR, Kosovo.
MOTS-CLÉS : *Parasites intestinaux, Microscopie optique, Afghanistan, RCA, Kosovo.*

INTRODUCTION

Intestinal parasitic infections belong to neglected diseases, one of the major health problems of the contemporary world[1]. It is estimated, that over 2 billion people are infected with intestinal parasites, and 5 billion live in areas where they are endemic[2]. The incidence of gastrointestinal parasitic diseases is exceptionally high in developing countries, where soil and water contamination, a limited number of households with access to safe drinking water sources, a large number of asymptomatic carriers, low standards of hygiene, and lack of health care result in the spread of orally transmitted infections[3, 4].

Between 2011 and 2015 the Polish Armed Forces (PAF) realized mandatory tasks in Asia, Africa and Europe. One of the elements of PAF military service was humanitarian aid for local patients with infectious diseases. The aim of the study was to present the results of parasitological examination performed among local residents in Afghanistan, Central African Republic and Kosovo in order to establish the prevalence of intestinal parasitic infections and to implement effective deworming strategies.

MATERIAL AND METHODS

Study population

The study covered three groups of inhabitants:
- Afghanistan (n=3,146): patients from the Ghazni Provincial Hospital, students attending high schools in Ghazni Province, soldiers from the Afghan National Army,
- Central African Republic (n=3,209): Monassao and Bagandou inhabitants of the Pygmy origin, residents of Bangui and Bagandou,
- Kosovo (n=632): students of primary schools in Kaçanik Province.

Each patient provided 1-3 stool samples, which were fixed in 10% formalin and transported to Poland, where they were pooled and examined using three different diagnostic methods in light microscopy for the presence of nematode, cestode, trematode and protozoan infections. All of the infected patients from Afghanistan, Central African Republic and Kosovo received recommended antiparasitic treatment.

Laboratory procedures

Stool examination was performed by means of three different testing methods by light microscopy (direct smear, Fülleborne's flotation and decantation in distilled water)[5, 6]:

Direct smear in Lugol's solution

Approx. 2 mg of stool was collected with a glass rod and applied onto a slide, a drop of Lugol's solution was added and the material was smeared over a 4 cm^2 surface. Next, a cover slide was placed on top of the preparation and the material was examined microscopically under correct magnification objective (x10, then x40).

Fülleborn's flotation

Approx. 2 g of stool was mixed with saturated NaCl solution in a test tube. Next, NaCl solution was added to the top of the tube. A cover slide was placed on the top of the tube and in contact with the suspension. After 30 minutes the cover slide was removed with tweezers and placed the wet side down on a slide. The preparation was ready for microscopic examination (objective x10 magnification).

Decantation with distilled water

Approx. 2 g of stool was mixed thoroughly with a small amount of water in a test tube. Next, water was added to the top of the tube. After 30 minutes the supernatant was decanted and another portion of water was added. This procedure had been repeated until clear supernatant was obtained, generally three to four times. The sediment was then placed on a slide and stained with Lugol's solution for microscopic examination (objective x40 magnification).

Statistical analysis

All statistical calculations have been performed using the statistical suite StatSoft Inc. (2011) STATISTICA version 10.0. www.statsoft.com (SN JGNP3087539302 AR-E) and Excel. The qualitative variables were presented with the use of count and percentage. P=0.05 was assumed statistically significant for all calculations.

RESULTS

Infections with intestinal parasites were detected in 1,225 of the tested Afghans (prevalence of 38.9%) in the period 2011-2014, with the most common *Ascaris lubricoides* (19.6%), *Giardia intestinalis* (15.3%), and *Hymenolepis nana* (6.6%) (Table 1).
Intestinal parasitic infections were detected in 2,231 of the tested inhabitants of Central African Republic (prevalence of 69.5%) in the period 2014-2015, with the most common *Ancylostoma duodenale/Necator americanus* (40.0%), *Ascaris lumbricoides* (36.5%), and *Giardia intestinalis* (22.5%) (Table 2).

Intestinal parasitic infections were detected in 76 of the tested inhabitants of Kosovo (prevalence of 12.0%) in the period 2015-2016, with the most common *Giardia intestinalis* (10.0%) (Table 3).

The infected patients received recommended antiparasitic medicines (Table 4) delivered for local medical centers, financed by the Polish Ministry of National Defence.

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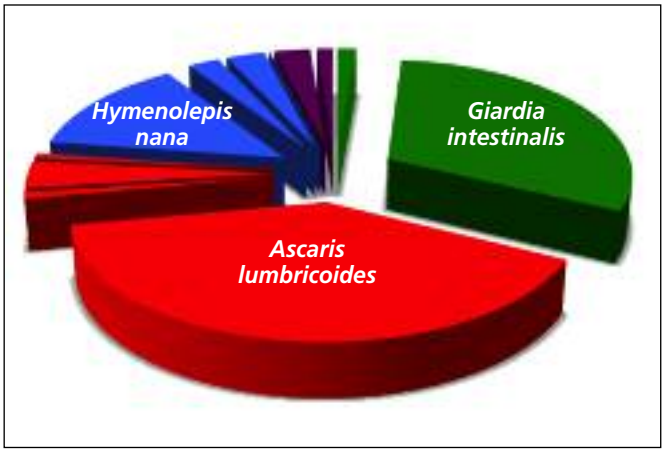
Table 1: Intestinal parasitic infections in inhabitants of Afghanistan in the period 2011-2014 (n=3146).

Intestinal parasites	No. of infections (n=1,553)	% of infected patients (n=1,225)	% of tested patients (n=3,146)
Nematodes	**698**		
Ascaris lumbricoides	617	50.4	19.6
Enterobius vermicularis	57	4.6	1.8
Ancylostoma duodenale/Necator americanus	14	1.1	0.5
Strongyloides stercoralis	7	0.6	0.2
Trichuris trichiura	2	0.2	0.06
Trichostrongylus spp.	1	0.1	0.03
Cestodes	**292**		
Hymenolepis nana	209	17.1	6.6
Hymenolepis diminuta	37	3.0	1.2
Taenia spp.	45	3.6	1.4
Diphyllobothrium latum	1	0.1	0.03
Trematodes	**61**		
Dicrocoelium dendriticum	42	3.4	1.3
Fasciola hepatica	19	1.6	0.6
Protoza	**502**		
Giardia intestinalis	480	39.2	15.3
Entamoeba histolytica s.l.	22	1.8	0.7
Number of infected patients	**1,225**	**100.0**	**38.9**

Figure 1: Parasite distribution in inhabitants of Afghanistan in the period 2011-2014 (n=3146).



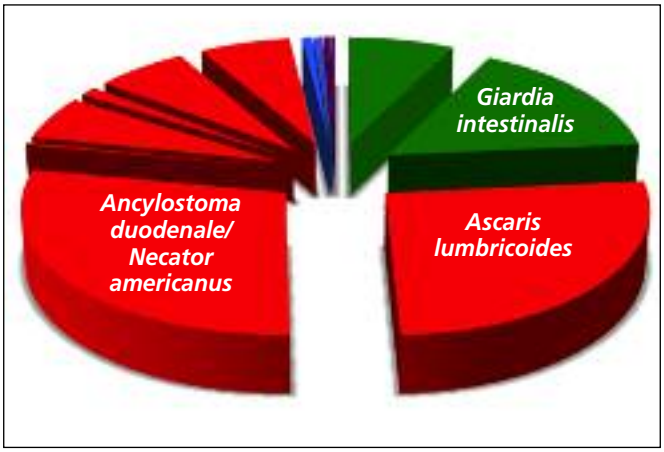
DISCUSSION

In the developing world, mass deworming is usually carried out by administering the WHO-recommended drugs[7]. As a rule, the World Health Organization recommends the administration of single doses albendazole or mebendazole[8]. This strategy is primarily aimed at the eradication of some nematode infections but in case of cestode, trematode and protozoan infections can be ineffective. Additionally, the large-scale administration of antihelminthics might result in the development and spread of drug resistant nematodes[9], which is already a significant problem in veterinary medicine[10]. Selected studies pointed, that the administration of a single dose of mebendazole lacked efficacy against *Ancylostoma/Necator* infections among schoolchildren in Zanzibar[11] and Vietnam[22]. Keiser and Utzinger[9] reported high cure rates of single dose mebendazole and albendazole for *Ascaris lumbricoides* (90%) and low cure rates in the same dose of mebendazole for Trichuris trichiura (36%) and *Ancylostoma/Necator* (15%). In Rwanda, a national survey among more than

Table 2. Intestinal parasitic infections in inhabitants of Central African Republic in the period 2014-2015 (n=3209).

Intestinal parasites	No. of infections (n=4,505)	% of infected patients (n=2,231)	% of tested patients (n=3,209)
Nematodes	**3359**		
Ancylostoma duodenale/Necator americanus	1283	57.5	40.0
Ascaris lumbricoides	1170	52.4	36.5
Trichostrongylus spp.	292	13.1	9.1
Trichuris trichiura	287	12.9	8.9
Strongyloides stercoralis	261	11.7	8.1
Capillaria spp.	53	2.4	1.6
Enterobius vermicularis	13	0.6	0.4
Cestodes	**41**		
Hymenolepis nana	30	1.3	0.9
Hymenolepis diminuta	9	0.4	0.3
Taenia spp.	2	0.1	0.1
Trematodes	**1058**		
Schistosoma mansoni	14	0.6	0.4
Dicrocoelium dendriticum	14	0.6	0.4
Fasciola hepatica	19	0.9	0.6
Protoza	**47**		
Giardia intestinalis	723	32.4	22.5
Entamoeba histolytica s.l.	335	15.0	10.4
Number of infected patients	**2,231**	**100.0**	**69.5**

Figure 2: Parasite distribution in inhabitants of Central African Republic in the period 2014-2015 (n=3209).

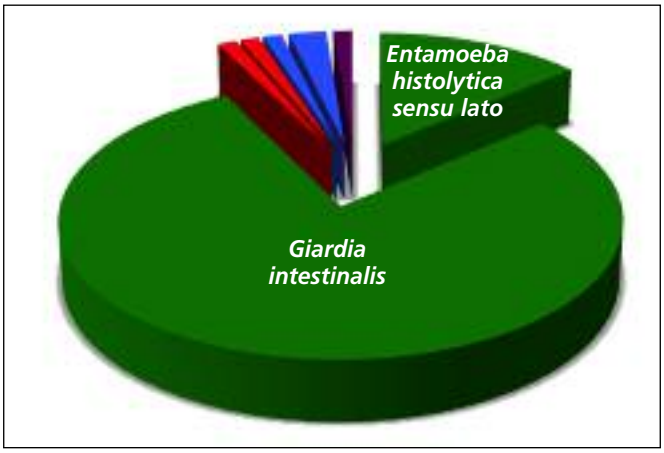


8000 school children in 30 districts found an overall soil transmitted helminths prevalence of 66%. A repeat survey conducted one year after the standard deworming found a reduction in ascariasis and trichuriasis prevalence by 14%, and increase in ancylostomiasis/necatoriasis prevalence by 72%[13]. World Health Organization gave particular emphasis to deworming in specific age groups, especially school age children. In 2001 the World Health Assembly declared the goal of ensuring that 75% of all school children in endemic areas should receive treatment. Pharmaceutical companies have donated treatment specifically for deworming of children, with 50 mg mebendazole treatments a year from Johnson & Johnson in 2007, rising to 200 mg in 2010, and 400 mg treatments of albendazole from GlaxoSmithKline in 2012[8]. The large donation of 600 million doses per year was announced in the London Declaration (a declaration on neglected tropical diseases was based in part on a new WHO roadmap) in 2012[14]. Unfortunately, deworming campaigns launched

Table 3. Intestinal parasitic infections in inhabitants of Kosovo in the period 2015-2016 (n=632).

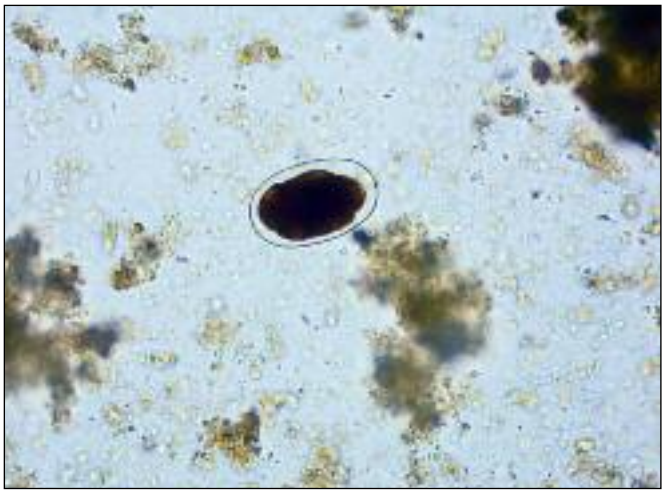
Intestinal parasites	No. of infections (n=80)	% of infected patients (n=76)	% of tested patients (n=632)
Nematodes	**2**		
Ascaris lumbricoides	1	1.3	0.15
Enterobius vermicularis	1	1.3	0.15
Cestodes	**3**		
Hymenolepis nana	1	1.3	0.15
Taenia spp.	2	2.6	0.3
Trematodes	**1**		
Fasciola hepatica	1	1.3	0.15
Protoza	**74**		
Giardia intestinalis	63	82.9	10.0
Entamoeba histolytica s.l.	11	14.5	1.7
Number of infected patients	**76**	**100.0**	**12.0**

Figure 3: Parasite distribution in inhabitants of Kosovo in the period 2015-2016 (n=632).



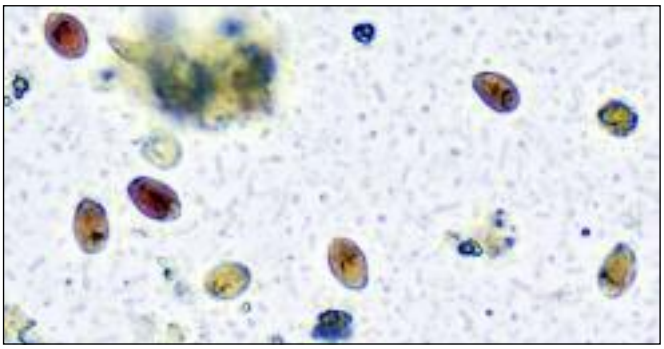
Picture 1: Ascaris Lumbricoides.
Source: Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, Poland.

Picture 2: Ancylostoma duodenale/Necatoc americanus.
Source: Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, Poland.



Picture 3: Strongyloides stercoralis.
Source: Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, Poland.

Picture 4: Giardia intestinalis.
Source: Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, Poland.



Picture 5: Hymenolepis nana.
Source: Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, Poland.

Picture 6: Schistosoma mansoni.
Source: Department of Epidemiology and Tropical Medicine, Military Institute of Medicine, Poland.

Table 4. Schedule of treatment for patients with selected intestinal parasitic infections.

Intestinal parasites	Treatment
Nematodes	
Ascaris lumbricoides ***Trichuris trichiura*** ***Trichostrongylus spp.*** ***Necator americanus/*** ***Ancylostoma duodenale***	albendazole 400 mg orally once (>2 years)
Enterobius vermicularis	albendazole 400 mg orally once (>2 years); treatment repetition after 2 weeks
Strongyloides stercoralis	ivermectin 200 µg/kg orally once a day for 2 days or albendazole 400 mg orally 2 times a day for 10-14 days
Cestodes	
Hymenolepis nana ***Hymenolepis diminuta***	praziquantel 25 mg/kg orally once
Taenia spp. ***Diphyllobothrium latum***	praziquantel 5-10 mg/kg orally once
Trematodes	
Schistosoma mansoni	praziquantel 20 mg/kg orally 2 times a day for one day
Dicrocoelium dendriticum	praziquantel 25 mg/kg orally 3 times a day for one day
Fasciola hepatica	triclabendazole 10 mg/kg orally once

Table 4. Schedule of treatment for patients with selected intestinal parasitic infections.

PROTOZA	
Giardia intestinalis	metronidazole 250 mg orally 3 times a day for 5-7 days or 500 mg orally 2 times a day for 5 days (adults and children >12 years); 250 mg orally 2 times a day for 5 days (10-12 years); 125 mg orally 3 times a day for 5 days (6-10 years); 125 mg orally 2 times a day for 5 days (2-5 years); children <2 years 1 x 5 mg/kg for 5 days
Entamoeba histolytica	
Intestinal colonisation (asymptomatic carrier)	paromomycin – 8-12 mg/kg orally 3 times a day for 7 days
Amebic colitis	metronidazole – 750 mg orally 3 times a day for 10 days (adults and children >12 years); children <12 years 30-50 mg/kg in 3 doses for 10 days
Amebic liver abscess	metronidazole – 750 mg orally or i.v. 3 times a day for 10 days (adults and children >12 years); children <12 years 30-50 mg/kg in 3 doses for 10 days
Source: Kappagoda S., Singh U., Blackburn B.G. Antiparasitic Therapy. Mayo Clinic Proceedings 2011; 86(6): 561–583	

in the developing countries of some regions in the last years have not been successful. For instance, only 0.69% school age children in the Eastern Mediterranean Region (North Africa, Middle East and Central Asia) received preventive antiparasitic therapy (albendazole or mebendazole)[15]. One of the main reasons why such campaigns end in failure is unstable geopolitical situation and local military conflicts. Because of that humanitarian aid is often provided by the international peace or stabilization forces and national military contingents stationing in some countries, e.g. the military health service of the Polish Armed Forces conduct parasitological screening in direction of presence of intestinal parasitic infections among local population residing Afghanistan, Central African Republic and Kosovo. The results obtained by the Polish military health service were comparable with results of previous studies carried out in the same areas. The first parasitological study into the occurrence of intestinal parasites in Afghanistan was carried out in Afghanistan by medical corps supporting the German Armed Forces deployed to Kabul on the Enduring Freedom operation. The stool specimens were collected from Afghan workers at the beginning of employment and sent to the Central Institute in Koblenz (Germany) using the Biosepar transport medium and examined microscopically. Of 217 Afghans employed on the international military base in Kabul (translators, dining facilities and cleaning personnel) 60.4% were found to be infected with helminths and pathogenic or non-pathogenic protozoa. The most commonly detected intestinal parasites were *Ascaris lumbricoides* (22.1%), *Entamoeba histolytica sensu lato* (5.1%) and *Giardia intestinalis* (3.2%)[16].

In 1984, a major documented screening study was conducted among inhabitants of Central African Republic to determine the prevalence of infections with intestinal parasites. Of 3352 study subjects, patients hospitalized in the country's capital, Bangui, 1570 were found to be infected with intestinal parasites (46.8%). The most commonly detected intestinal pathogens were *Ancylostoma duodenale* (26.7%), *Schistosoma mansoni* (20.8%), *Entamoeba histolytica* (18.2%). Multiple infections were common (an infection by more than one parasite species in a single patient)[17]. Recent studies conducted in Central African Republic among Aka Pygmies (involving the same ethnic group which had been tested in the present study) showed similar, high infection rates. Stool samples were collected from 62 men using ParaPak vials with formalin. 56 (90.3%) of the study participants were infected with hookworms (including *Ancylostoma duodenale and Necator americanus*), 31 (50%) with *Trichuris trichiura*, and 28 (45.2%) with *Ascaris lumbricoides*[18].

So far, the only research study into the prevalence of intestinal parasites in Kosovo was carried out on a small sample of patients from Kosovska Mitrovica in 2001. A total of 45 children with diarrheas were tested, 40% were found to be infected with *Giardia intestinalis* protozoa[19]. A study conducted by the present authors between 2015 and 2016 among 632 residents of southern Kosovo (Kaçanik district) demonstrated parasitic infections in 12% of the study participants, mainly with *Giardia intestinalis* (10.0%) and *Entamoeba histolytica sensu lato* (1.7%).

Owing to high prevalence of multiple infections (nematodes, cestodes, trematodes, protozoa) in the Afghan community and the dominance of protozoan infections in residents of Kosovo, it seems that a mass deworming campaign with a single-dose chemotherapy (albendazole 400 mg or mebendazole 500 mg) may prove ineffective in eradicating intestinal parasites in the local population. The effectiveness of deworming may be enhanced by increasing the dosage of albendazole (400 mg for three-five days)[20, 21] or mebendazole (600 mg for three days)[22] or usage of complex therapy

(albendazole, metronidazole, praziquantel). Before initiating deworming in the developing countries it would be considerable to perform parasitological examination in order to define the prevalence and identify the most common species of parasites in the local population.

Deworming campaigns should be conducted on a regular basis, at least once a year, due to a high risk of reinfection among local populations.

Limitations of the study

Stool examination was made four-eight weeks after collection and transfer of biological material fixed in 10% formalin to Poland. This prolonged time of examination and fixation might lead to underestimation of some intestinal parasitic infections. For protozoa only cysts were taken into account.

CONCLUSIONS

The results revealed significant differences between parasitic infections distribution in each of the countries. Large variety of intestinal parasites found in Afghanistan and the dominance of protozoa in Kosovo may require to verify the WHO preventive deworming procedures (single doses of 400 mg albendazole or 500 mg melbendazole). Before initiating deworming in the developing countries it would be considerable to perform parasitological examination in order to define the prevalence and identify the most common species of parasites in the local population.

ABSTRACT

Objective. Between 2011 and 2015 the Polish Armed Forces (PAF) realized mandatory tasks in Asia, Africa and Europe. One of the elements of PAF military service was humanitarian aid for local patients with infectious diseases. The aim of the study was to present the results of parasitological examination performed among local residents in Afghanistan, Central African Republic and Kosovo in order to establish the prevalence of intestinal parasitic infections and to implement effective deworming strategies.

Material and Methods. The study covered three groups of inhabitants: Afghanistan (n = 3,146, patients from the Ghazni Provincial Hospital, students attending high schools in Ghazni Province, soldiers from the Afghan National Army), Central African Republic (n = 3,209, Monassao and Bagandou inhabitants of the Pygmy origin, residents of Bangui and Bagandou), and Kosovo (n = 632, students of primary schools in Kaçanik Province). Each patient provided 1-3 stool samples, which were fixed in 10% formalin and transported to Poland, where they were pooled and examined using three different diagnostic methods in light microscopy (direct smear, Fülleborn's flotation and decantation in distilled water).

Results. Intestinal parasitic infections were detected in 1,225 of the tested Afghans (prevalence 38.9%, the most common *Ascaris lumbricoides* 19.6%, *Giardia intestinalis* 15.3%), 2,231 inhabitants of Central African Republic (prevalence 69.5%, the most common *Ancylostoma duodenale/Necator americanus* 40.0%, *Ascaris lumbricoides* 36.5%), and 76 inhabitants of Kosovo (prevalence 12.0%, the most common *Giardia intestinalis* 10.0%). The infected patients received recommended antiparasitic treatment.

Conclusions. The results revealed significant differences between parasitic infections distribution in each of the countries. Large variety of intestinal parasites found in Afghanistan and the dominance of protozoa in Kosovo may require to verify the WHO preventive deworming procedures (single doses of 400 mg albendazole or 500 mg melbendazole). Before initiating deworming in the developing countries it would be considerable to perform parasitological examination in order to define the prevalence and identify the most common species of parasites in the local population.

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