

Detection of *Giardia intestinalis* infections in Polish soldiers deployed to Afghanistan

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ABSTRACT

Background: Members of the Polish Military Contingent (PMC) have been stationed in Afghanistan since 2002. They typically serve in areas characterised by low standards of sanitation which often leads to the development of food- and waterborne diseases. The aim of the study was to evaluate the prevalence of *Giardia intestinalis* infections among Polish soldiers deployed to Afghanistan. The research study was conducted as part of a programme for prevention of parasitic diseases of the gastrointestinal tract run by the Polish Armed Forces.

Materials and methods: The study was carried out in August 2011; it involved 630 asymptomatic Polish soldiers serving in the Forward Operational Base (FOB) Ghazni in eastern Afghanistan. Stool specimens obtained from members of the PMC were first tested in FOB Ghazni (detection of *Giardia intestinalis* by Rida Quick *Giardia* immunochromatographic tests and Ridascreen *Giardia* immunoenzymatic tests – single samples). Next, the same biological material and two other faecal specimens fixed in 10% formalin were transported to the Military Institute of Medicine in Poland, where they were tested for *Giardia intestinalis* under light microscopy (direct smear, decantation in distilled water).

Results: Parasitological tests performed under light microscopy showed that 2.7% (17/630) of the study group were infected with *Giardia intestinalis*. Some of these results were confirmed by immunochromatographic tests (6/630). In contrast, immunoenzymatic tests (ELISA) demonstrated a significantly higher detection rate reaching 18.1% (114/630). Immunoenzymatic tests confirmed all the positive results given by light microscopy and by immunochromatographic tests.

Conclusions: The prevalence rate of *Giardia intestinalis* infections in Polish soldiers deployed to Afghanistan was found to be high. Microscopic methods exhibit low sensitivity and therefore may result in the underestimation of the true parasite prevalence. Immunoenzymatic tests (ELISA) showing a much higher sensitivity in comparison to light microscopy and immunochromatographic tests ought to be applied in screening for intestinal protozoan infections in areas characterised by harsh environmental conditions.

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Key words: *Giardia intestinalis*, Polish soldiers, Afghanistan, diagnostics

INTRODUCTION

Giardia intestinalis is a protozoan parasite which often infects mammals including humans. *Giardia* infections occur worldwide [1]; it is the most commonly identified intestinal parasite both in developing and developed countries [2]. In the developed world, the prevalence of giardiasis is 2% to

5%, while in developing countries *Giardia* prevalence reaches 20% to 30% [3]. *Giardia* infections may cause intestinal malabsorption presenting with diarrhoea but can also be asymptomatic [4]. Transmission occurs directly by ingestion of intermittently shed and immediately infectious *Giardia* cysts. Additionally, contaminated water or food may become



a source of infection [5, 6]. *Giardia* cysts are the environmentally stable stage and are resistant to inactivation by drinking water disinfectants, remaining viable for up to 2 months [7]. The diagnosis of giardiasis is initially based on clinical signs and symptoms and confirmed by the presence of cysts and trophozoites in stool samples [8]. The gold standard for the diagnosis is still microscopic examination [9], but detection of antigen in stool by enzyme immunoassay (enzyme-linked immunosorbent assay [ELISA]) is a more sensitive method, recently more and more frequently used [10, 11]. A definitive diagnosis of *Giardia* infection may require repeated microscopic stool examinations and stool immunoassays [12].

Despite significant progress in laboratory diagnostics and treatment methods, intestinal protozoan infections remain a major health problem worldwide, especially in the Third World countries. There are a number of factors which facilitate the spread of protozoan infections, including poor sanitation, lack of medical care, mass migration, and the presence of hosts in ecosystems (reservoirs of parasites) [13–15]. Afghanistan provides an excellent example of a developing country characterised by high rates of invasive gastrointestinal tract diseases. The author's study, carried out among patients with internal complaints treated in the Ghazni Provincial Hospital, eastern Afghanistan in 2012, found that 49.4% of the examined children and 24.3% of adults were infected with intestinal parasites, predominantly with ascariasis, giardiasis and hymenolepiasis [16].

The Afghan healthcare system is heavily dependent on humanitarian aid provided by international non-governmental organisations (NGOs). The shortage of medical staff at all levels of the healthcare system hampers the implementation of epidemiological surveillance and makes it difficult to reduce the number of the sick and carriers. Water contamination is widespread in Afghanistan and therefore no more than 31% of Afghan households have access to uncontaminated drinking water, and only 5–7% have access to toilet facilities which meet the sanitary requirements [17]. Over 50% of Afghans are estimated to be chronically malnourished. Cases of invasive diseases found among Afghans are rarely laboratory-confirmed [18].

Members of the Polish Military Contingent (PMC) have been stationed in Afghanistan since 2002. The areas of their operation are characterised by poor standards of sanitation and this often leads to the development of food- and waterborne diseases. In order to prevent the spread of diseases among Polish soldiers deployed on overseas missions, a specially designed programme for prevention of parasitic diseases of the gastrointestinal tract had been implemented in the Polish Armed Forces. The programme helped maintain the epidemiological surveillance and eliminate protozoan infections from the military environment [19].

The aim of the study was to evaluate the prevalence rates of *Giardia intestinalis* infections in Polish soldiers deployed to Afghanistan. The research study was conducted as part of the above-mentioned prevention programme.

MATERIALS AND METHODS

STUDY POPULATION

The study was conducted in August 2011 and involved 630 soldiers from the PMC deployed to the Forward Operational Base (FOB) Ghazni in eastern Afghanistan. The soldiers studied were aged 22–48; the mean age being 31.4 years (< 25 years – 16.0%; 26–35 years – 59.8%; 36–45 years – 21.9%; > 46 years – 2.2%), they represented three different corps, i.e. privates – 49.8%, non-commissioned officers – 34.8%, officers – 15.4%. All study participants were in a good general condition, which was a prerequisite for the military service abroad (confirmed by medical tests and examination by a medical board before deployment to Afghanistan). The subjects enrolled in the study were patrol, sentry and operational troopers who frequently had contact with the local people and often consumed the local food. The study involved soldiers who had been serving in Afghanistan for a period of at least 4 months and had never been diagnosed or treated for parasitic infections before.

SAMPLE COLLECTION

Stool examination was conducted in two stages. The first stage was carried out in FOB Ghazni and included collection of single samples of fresh stool from members of the PMC Afghanistan. A portion of the sample was used for the detection of *Giardia intestinalis* by Rida Quick Giardia immunochromatographic test and Ridascreen Giardia immunoenzymatic tests. The rest of the first sample and two other stool specimens collected at 1 to 2-day intervals were fixed in 10% formalin and then transported to the Military Institute of Medicine in Poland, where they were tested for *Giardia intestinalis* by light microscopy using two diagnostic methods (direct smear, decantation in distilled water). A total of 630 immunochromatographic tests, 630 immunoenzymatic tests (ELISA) (single tests), and 3,780 examinations under light microscopy (two methods × three samples from each of the studied soldiers) were performed.

LABORATORY PROCEDURES

Single fresh stool samples obtained from each patient were used to detect *Giardia* antigen by immunochromatographic Rida Quick test (R-Biopharm AG, Darmstadt, Germany) following the manufacturer's instructions. The Rida Quick Giardia is a test for the qualitative determination of *Giardia intestinalis* in stool samples (a single-step lateral-flow test based on the principle of chromatography in which specific

anti-*Giardia* antibodies bind to latex particles and adhere to the surface of the test membrane producing a colour band on the strip if the test result is positive). The method has its limitations, i.e. a negative test result does not rule out the risk of *Giardia intestinalis* infection but may be associated with insufficient faecal excretion of the antigen. Next, the same single fresh stool samples were used to detect *Giardia* antigen by Ridascreen enzyme immunoassay (ELISA) test (R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's instructions. One positive and two negative controls were used at each run. Optical density (OD) was measured with an automatic microplate spectrophotometer. A positive result was defined as an OD reading 10% over the cut-off value (negative control OD +0.15).

A negative test result does not rule out the risk of *Giardia intestinalis* infection but may be associated with too little amount of antigen in the stool sample (100% sensitivity, 99.6% specificity, 95.5% positive predictive value [PPV], and 100% negative predictive value [NPV] according to the manufacturer).

Next, 2 to 4 weeks after performing immunochromatographic and immunoenzymatic tests, three stool samples obtained from each patient, fixed with 10% formalin were used to prepare a wet mount and tested for the presence of *Giardia intestinalis* by direct smear and decantation in distilled water (both counterstained with Lugol's solution). All samples were examined under light microscopy using $\times 10$, then $\times 40$ (direct smear), and $\times 40$ (decantation) objective magnification.

STATISTICAL ANALYSIS

The statistical analyses have been performed using the statistical suite StatSoft Inc. (2014) STATISTICA (data analysis software system) version 12.0. www.statsoft.com and Excel. The qualitative variables were presented with the use of count and percentage. Chi-squared tests for independence were used for qualitative variables. The quantities variables were characterised by the arithmetic mean of standard deviation and max/min (range). Statistical significance of differences between two groups was processed with the t-Student test or U Mann-Whitney test. In all the calculations the statistical significance level of $p = 0.05$ has been used.

RESULTS

The study involved 630 soldiers serving in FOB Ghazni, eastern Afghanistan and was conducted in August 2011. Parasitological tests for *Giardia intestinalis* were performed using three different diagnostic methods. A stool sample was considered *Giardia*-positive if at least one test result was positive. A total of 630 stool samples were tested. Immunochromatographic tests produced positive results in 6 of 630 samples tested; enzyme immunoassay (ELISA) tests gave 114 positive results and light microscopy techniques (direct smear, decantation in distilled water) produced positive results in 17 of all the samples examined.

All six samples revealed as positive by immunochromatographic tests were also positive using light microscopy and ELISA. All 17 samples revealed as positive by light microscopy were also positive using ELISA. Among 114 samples revealed as positive by ELISA, 97 were negative using light microscopy and 108 were negative using immunochromatographic tests.

The sensitivity, specificity, PPV and NPV results for *Giardia intestinalis* infections are presented in Table 1.

In total, 114 of 630 study participants (18.1%) were found to be infected with *Giardia intestinalis*. The percentage distribution of *Giardia* infection was as follows: < 25 years – 21.0%; 26–35 years – 63.2%; 36–45 years – 14.9%; > 46 years – 0.9%. The mean age of the infected soldiers was 29.7 years, while the mean age of non-infected soldiers was 31.8 years (range 22–48 years). Statistical analysis showed that soldiers infected with *Giardia intestinalis* were significantly younger in comparison to non-infected soldiers ($p = 0.0001$). The distribution of infection by rank (privates – 53.5%, non-commissioned officers – 41.2%, officers – 5.3%) showed that *Giardia* infections were more commonly found in soldiers in lower ranks.

DISCUSSION

Soldiers deployed on military operations in the Third World countries are experiencing difficult sanitary conditions and therefore are at a higher risk of developing infectious diseases. Medical services supporting overseas operations need to pay particular attention to intestinal parasitic infections since these are extremely widespread and can be easily transmitted through the oral-faecal route.

Table 1. Accuracy of three diagnostic methods for the detection of *Giardia intestinalis* infections (n = 630)

Diagnostic methods for the detection of <i>Giardia intestinalis</i>	No. of infections	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Light microscopy (direct smear, decantation)	17	14.9%	100.0%	100.0%	85.1%
Rida Quick Giardia test (immunochromatographic)	6	5.3%	100.0%	100.0%	82.7%
Ridascreen Giardia test (ELISA)	114	100.0%	100.0%	100.0%	100.0%

PPV – positive predictive value; NPV – negative predictive value

These conditions are often asymptomatic and may take a chronic form [20]. The examination of Polish soldiers deployed to Chad and Central Africa in the period 2008–2009 demonstrated a high rate of intestinal parasitic infections, predominantly *Giardia intestinalis* infections (55/247, 22.3%) [21]. The present study conducted among members of the PMC deployed to Afghanistan demonstrated a similar prevalence of *Giardia intestinalis* infections (114/630, 18.1%).

A majority of research reports dealing with intestinal protozoan infections present the results obtained by microscopic examination only. This is associated with low costs of the light microscopy but also the opportunity it gives to detect a wider variety of parasites in the same specimen [22]. However, lower sensitivity of microscopic methods, much depending on a diagnostician's knowledge and experience, may result in the underestimation of the true parasite prevalence. The results of microscopic examination are not only influenced by the experience of laboratory specialists but also the number of faecal samples examined [23] and the application of appropriate concentration techniques (in accordance with the procedures recommended by the Clinical and Laboratory Standards Institute) [24]. Light microscopy may exhibit lower sensitivity due to the irregular presence of protozoa in stool or their low number in the faecal sample [25]. According to Noor et al. [26] microscopic examination of a single stool specimen has 46% sensitivity only. Hence, at least three faecal samples have to be taken and examined over a 3–5 day period to achieve 94% [27, 28], or according to other authors 50–70% [29] sensitivity in diagnosing positive *G. intestinalis* cases. For this reason, more sensitive diagnostic methods are recommended for the diagnosis of giardiasis, for instance (ELISA) in which the detection of *Giardia intestinalis* is based on the presence of a specific antigen (glycoprotein GSA 65) in cysts and trophozoites [30]. The advantage of enzyme immunoassays over microscopy is their higher sensitivity for protozoa in single faecal samples [31].

The present study used three diagnostic methods for the detection of protozoa, i.e. light microscopy, immunochromatographic tests and immunoenzymatic tests (ELISA). The overall prevalence of *Giardia intestinalis* infection was 2.7% for light microscopy, 0.95% for immunochromatographic tests, and 18.1% for ELISA. The study results thus demonstrated that enzyme immunoassay tests exhibited the highest sensitivity, given a similar specificity of all the three testing methods. The results of other studies comparing the sensitivity of light microscopy and immunoenzymatic tests in detecting intestinal protozoan infections have also confirmed a higher sensitivity of enzyme immunoassays [11, 26]. The present study used three stool samples for mi-

croscopic examination (and only one for immunochromatographic and immunoenzymatic tests); however, microscopic examination was distant in time from sample collection (2 to 4 weeks after stool specimens had been taken) and the biological material was fixed in 10% formalin, which might have led to the underestimation of the true prevalence of parasitic infections.

The prevalence of *Giardia intestinalis* infections is closely associated with the socioeconomic status of a given country. The author's study conducted in eastern Afghanistan between 2011 and 2014 among 3,146 residents of the Ghazni province demonstrated giardiasis prevalence of 15.5%; the prevalence rates were similar in three different study groups: soldiers (n = 110; 13.6%), hospital patients (n = 1167; 13.8%), and students of primary and secondary schools (n = 1869; 16.7%) [32]. Studies into the prevalence of *Giardia intestinalis* in developed countries have showed how high standards of sanitation and good hygiene practices may reduce the rates of the infection. Population-based studies in children conducted in European countries demonstrated the giardiasis prevalence of 0.8% in Italy (by light microscopy) [33], 1.3% in Great Britain (by immunofluorescence microscopy) [34] and of 1.5% in Germany (by immunofluorescence microscopy) [35]. In Poland, a country with a population of approximately 38 million inhabitants, a total of 1,742 cases of giardiasis were detected in 2015 (morbidity rate 4.53/100,000 inhabitants) against 1,872 cases in 2014 (4.86/100,000) [36]. However, according to Bajer [37], the rates of *Giardia intestinalis* in the Polish population reported by the National Institute of Public Health, are significantly underestimated.

LIMITATIONS OF THE STUDY

Stool examination in light microscopy was performed 2–4 weeks after sample collection and their transfer (fixed in 10% formalin) from Afghanistan to Poland. The fact that the examination was distant in time from sample collection and the biological material obtained was formalin-fixed might have resulted in the underestimation of the true prevalence of parasitic infections.

CONCLUSIONS

The prevalence of *Giardia intestinalis* infections in Polish soldiers deployed to Afghanistan was found to be high. Low sensitivity of light microscopy could result in the underestimation of the true parasite prevalence. Immunoenzymatic diagnostics (ELISA) exhibiting a much higher sensitivity in comparison to light microscopy and immunochromatographic tests should be used in screening for intestinal protozoan infections in areas characterised by harsh environmental conditions.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest in relation to this article.

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