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Review article

Contamination of soil with eggs of geohelminths *Ascaris* spp., *Trichuris* spp., *Toxocara* spp. in Poland – potential source of health risk in farmers

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ABSTRACT. Soil is one of the most commonly occurring sources of biological hazards in the environment. It is a reservoir of many pathogens and an important factor in the environmental transmission of parasites. The aim of the article is to discuss the general degree of parasitic contamination of agriculturally used soil in Poland from the aspect of potential health risk for farmers. Parasitic studies of soil conducted to-date in Poland demonstrate that it is considerably contaminated. The presence of the eggs of geohelminths *Ascaris* spp., *Toxocara* spp., and *Trichuris* spp was most often observed. The main cause of contamination of agriculturally used soil are fertilizers, sewage sludge, as well as animal faeces. The presence of invasive forms of parasites in the environment creates the risk of infection in humans due to direct or indirect exposure resulting from the way of performing work. In Poland, the frequency of infections with nematodes of the genera *Ascaris* spp., *Trichuris* spp., *Toxocara* spp. in humans has not been recognized. Considering the high percentage of positive results obtained in parasitic examinations of soil it may be presumed that there is still a real threat to human health, including population groups engaged in agricultural production.

Keywords: geohelminths, parasite eggs, contamination of soil, farmers

Introduction

Soil is a surface, biologically active layer of the lithosphere. It enters into the composition of all terrestrial ecosystems and occupies a special position in the natural environment. It participates, among other things, in the production and decomposition of biomass, the cycle of elements and energy in ecosystems, as well as the accumulation and protection of water resources. It creates a habitat for many organisms participating in the course of soil processes and maintenance of food safety, the taxonomic and functional diversity of which is affected by the agricultural practices applied [1].

The main direction of using soil resources by humans is agricultural production, which includes activities in the area of plant and food production (field crops, horticulture, vegetable growing, fruit growing), as well as breeding and livestock rearing. In Poland, agricultural areas occupy the surface of approximately 14,600,000 ha, which is nearly 50% of the total territory of the country [2]. Farming and livestock rearing generate large amounts of organic waste, raw materials and by-products which, considering chemical composition, properties and high biological activity (activity of micro - organisms), require proper management with the simultaneous preservation of protection of the natural environment, food safety, and health of humans and animals [3,4]. A common direction in the use of agricultural waste is farming and reclamation of land, production of fertilizers and soil conditioners, and improvement of physico-chemical properties of agriculturally used soils [5]. Improper processing, use and storage of fertilizers produced from agricultural waste results in the biological contamination of surface and deep waters and soils. This leads to contamination of cultivated plants designed for consumption or livestock feed. Pathogenic microorganisms may be introduced into the soil, including bacteria

Salmonella spp., *Escherichia coli*, *Brucella* spp., *Chlamydia* spp., *Leptospira* spp., *Rickettsia* spp., *Mycobacterium* spp., protozoa of the genera *Eimeria* spp., *Giardia* spp., *Cryptosporidium* spp., as well as the eggs and larvae of parasitic nematodes [6,7]. The occurrence in soil of dispersive forms of some nematode species (geohelminths) is naturally connected with their development cycle. The eggs are excreted with the hosts' faeces into the external environment, where in optimum conditions of humidity, temperature, and oxygen availability, they undergo further development to an invasive form. Several species of geohelminths are of primary epidemiological importance, including: human roundworm *Ascaris lumbricoides*, pig roundworm *Ascaris suum*, whipworm *Trichuris trichiura,* dog roundworm *Toxocara canis* and feline roundworm *Toxocara cati*, with which humans are infected most often via the alimentary route, by contact with contaminated soil and consumption of contaminated vegetables and fruits. The eggs of the above mentioned nematodes are resistant to the effect of environmental factors and are characterized by long survival in the environment, while maintaining invasiveness (from 5–10 years). Several years of survival of invasive eggs may lead to their accumulation in the external environment.

The article discusses the sources and degree of contamination of agriculturally used soil in Poland, and the resulting potential health risk for people exposed to contact with soil in which the eggs of intestinal parasites of the genera *Ascaris* spp., *Trichuris* spp. and *Toxocara* spp. are present.

Sources and degree of parasitological contamination of soil in Poland

In Poland, studies of biological contamination of soil with the eggs of intestinal parasites from geohelminths group were carried out mainly in urban areas. The degree of parasitological contamination of soil collected from various public places, including sandboxes and playgrounds for children, recreation areas, parks and urban squares, was estimated at 41.4% – in Szczecin, 21.6% – in Lublin, 20% – in Wrocław, and 1.25–25% in Warsaw and its vicinity [8–11]. The majority of detected geohelminths eggs belonged to the genera *Toxocara* spp. and *Trichuris* spp., and the species *Toxascaris leonina*.

High contamination of agricultural land used for the cultivation of vegetables and fruits was observed in rural areas. In the Lublin Province, the eggs of geohelminths were detected in 88.5% of samples of soil from conventional farms, and in 32.8–42.16% of soil samples from farms run in the ecology system [12,13]. The data by Kłapeć (data unpublished) show that the contamination of soil with the eggs of intestinal parasites on conventional farms (56.9%) is nearly three times higher than in ecology farms (19.3%). At the same time, it was found that the samples of soil collected from vegetable gardens were most contaminated parasitologically (34.4%). Kłapeć et al. [14] observed the presence of the eggs of *Ascaris* spp. and *Toxocara* spp. in 18 out of 45 samples of soil used for greenhouse crops and under foil cover, simultaneously indicating a high level of parasitological contamination – 40%. Studies of the contamination of soil with the eggs of geohelminths were also carried out in rural areas located near Łódź, where small, family farms are engaged in the breeding of swine, cattle and poultry [15]. The eggs of *Ascaris* spp. were most frequently detected in the samples of soil from cultivated land (87.%) fertilized with liquid pig manure. In turn, the largest amount of the eggs of *Toxocara* spp. were found in samples of soil collected from home gardens (73.9%), to which both dogs and cats had an access. Studies of the samples of soil used for the cultivation of vegetables and fruits in the rural areas showed the degree of parasitological contamination on the level of nearly 25% (unpublished data). Similar to the studies by other researchers, the most often detected, and in large numbers, were dispersive forms of parasitic nematodes, the eggs of *Ascaris* spp., *Toxocara* spp. and *Trichuris* spp.

The presented results of studies by various researchers indicate a high parasitological contamination of soils in Poland, and thus a real problem of threat to public health. Based on the identified genera and species of geohelminths in the examined soil samples, it may be presumed that the type and degree of parasitological contamination of soil in urban and rural areas is associated with the occurrence of different sources of the spread of parasites in the environment. In the examined samples of soil collected in urban areas, the highest percentage was found of the eggs of *Toxocara* spp., which evidences the dominant contribution of carnivorous animals (dogs, cats) in the transmission of the parasite. In Katowice, the presence of the eggs of this species was confirmed in approximately 50% of the examined samples [16]. In rural areas,

the main source of parasitological contamination of agriculturally used soil were natural fertilizers (slurry, liquid manure, manure), organic and organic-mineral fertilizers, and soil conditioners produced from by-products of animal origin. It was confirmed that some agricultural practices are highly associated with the contamination of soil by parasite eggs [17]. Studies of organic fertilizers carried out during 2014–2015 at the Department of Parasitology and Invasive Diseases, National Veterinary Research Institute in Puławy, demonstrated that the percentage of positive samples containing the eggs of parasitic nematodes was on the level of 65%, including 89% of samples from biogas plant, 7.9% samples of fertilizers produced from sewage sludge, and 2.6% of samples from bio-composting plant [18]. In positive samples, the eggs of nematodes of the genera *Ascaris* spp. and *Trichuris* spp. were found, while the eggs of parasites of the genus *Toxocara* spp. were not detected. A serious risk of biological contamination is also associated with the agricultural use of sewage and sewage sludge with an unknown sanitary condition. The presence of the eggs of parasites of the genera *Ascaris* spp. (84.3%) and *Trichuris* spp. (17.2%) was confirmed in approximately 45% of fermented sewage sludge [19]. The application of a new, more effective method of detection of the eggs of intestinal parasites from sewage sludge showed a considerably higher degree of parasitological contamination – 99% [20]. The eggs of the genera *Ascaris* spp., *Trichuris* spp. and *Toxocara* spp. were found subsequently in 95%, 96% and 60% of samples of dehydrated sewage sludge collected from various municipal sewage treatment plants in Poland.

Wild animals are the reservoir of zoonotic parasitic nematodes. Developmental forms of parasites of the genera *Toxocara* spp., *Trichuris* spp., *Capillaria* spp., *Eimeria* spp., *Trichostongylus* spp. were detected in more than 66% of samples of faeces from boars, hares, deer and fallow deer inhabiting the areas of the Poznań Province [21]. Boars occurring in agriculturally used areas were most often infected with *Ascaris suum* (61.5%) and *Trichuris suis* (84.6%) [22]. It was confirmed that there is the possibility of transmission of parasites between boars and breeding pigs via soil fertilized with pig manure. Simultaneously, a study conducted on Polish pig farms demonstrated that the percentage of the occurrence of invasion with *Ascaris suum* and *Trichuris suis* was subsequently 28.6% and 21.4% [23]. A high percentage of infection of animals with *Trichuris* spp. (53.5%) was observed in the population of moose in north-eastern Poland, in the Biebrza wetlands [24]. Infected animals contribute to the spread and preservation of invasive forms of zoonotic species of parasites in the external environment. Moreover, an increase in the population of wild animals, fragmentation and shrinking of their habitation areas, as well as adjustment to life in the vicinity of human settlements, creates the possibility of biological contamination of soil and threat to human health, domestic and farm animals in rural areas [21].

Also, domestic animals which are the reservoir of *Toxocara* spp., participate in the parasitological contamination of soil. A coprological study of rural dogs in the Provinces of Warsaw and Kraków showed a high extensiveness of infections with intestinal parasites reaching from 0.9–22.2% [25]. The eggs of zoonotic geohelminths *Toxocara canis* and *Trichuris vulpis* were detected in 7.2% and 20.2% of samples of faeces, respectively. In turn, Michalczyk et al. [26], in their study found the eggs of *Toxocara canis* in more than 40% of samples of faeces in rural dogs. It is noteworthy that among the owners of animals in cities, the awareness of the necessity to observe the principles of prophylaxis and regular deworming of dogs and cats, justified the need for removing faeces from the environment, and is considerably higher than among the inhabitants of rural areas.

Legal regulations and methods of examination of the sanitary state of soil in Poland

In Poland, there is no obligation to conduct monitoring of the sanitary state of agriculturally used soil. Polish and EU regulations concern only issues related with the principles of production, storage, and admission to trading and use of organic fertilizers, and soil conditioners containing raw material of plant or animals origin, which may be the source of hazardous biological agents introduced into the soil environment, and may create a threat to health for humans and animals alike. The basic Polish legal act regulating the above-mentioned problems is the Act of 10 July 2007 in the matter of fertilizers and fertilization [27], supplemented by a series of executive acts, including the Regulation of the Minister of Agriculture and Rural Development of 16 April

2008 on the detailed manner of applying fertilizers and conducting training in their use [28], and the Regulation of the Minister of Agriculture and Rural Development of 18 June 2008 on the implementation of certain provisions of the Act on fertilizers and fertilization [29]. In the case of the use of by-products of animal origin or their derivatives for the production of fertilizers or soil conditioners, the relevant provisions of the veterinary law apply [30]. Considering the transmission of pathogenic agents along the food chain and the relationship between soil microflora and the microflora of plants cultivated on this soil, the applicable legal requirements contribute to the reduction of health risk to consumers [31]. The New Water Law [32] imposes a number of obligations on farmers associated with safe storage and application of natural fertilizers (slurry, liquid manure, manure), in order to protect water and soil against chemical and biological contamination. According to this Act, natural fertilizers cannot be applied on soils flooded with water, covered with snow, frozen to the depth of 30 cm, and on land where deep water is to a depth of less than 1.5 m, and during rainfall. It is prohibited to apply liquid natural fertilizers on soil located on slopes of more than 10% without plant cover, and during vegetation of plants designed for direct consumption by humans.

In Poland, the parasitological indicators belonging to the evaluation of the sanitary state of soil are live eggs of parasites belonging to nematodes of the genera *Ascaris* spp., *Trichuris* spp., *Toxocara* spp. [19]. The methods of their detection and determination in soil samples have been developed and contained in the Polish Standard No. PN-Z-19000-4:2001 [33], and in Methodological Guidelines (bacteriological-parasitological) for the sanitary evaluation of soil [34]. Detection of the presence of at least one invasive form of *Ascaris* spp., *Trichuris* spp., *Toxocara* spp. in the examined sample allows it to be considered as contaminated.

Effect of parasitological contamination of soil on human health

Human roundworm, *Ascaris lumbricoides* and whipworm *Trichuris trichiura,* are cosmopolitic nematodes that are parasitic in the human alimentary tract. They cause diseases such as ascariosis and trichuriosis. In humans, cases of infection with *Ascaris suum* and *Trichuris suis* were commonly observed occurring in pigs and boars, and also *Trichuris vulpis* occurring in canids [35–37]. The frequency of occurrence of ascariosis in Poland is estimated at 1–18%, in turn, the frequency of infection with whipworm in humans ranges from 2.3–25% [38]. In the majority of the infected people the course of invasion is asymptomatic. In the clinical image of ascariosis symptoms are distinguished related with the migration of larvae, invasion into the small intestine, and complications. The symptoms are non-specific, remind gastrointestinal diseases, allergies, cutaneous symptoms, infections of the upper airways, or bronchial asthma [39]. Infection with *Ascaris lumbricoides* in children and adults may affect the nervous system and occurrence of psychomotor disorders, such as motor and vocal tics, onychophagia, and hyperexcitability [40]. Similarly, the majority of cases of trichuriosis in humans have an asymptomatic course or a mild form. In persons with a massive invasion there may occur symptoms on the part of alimentary system, including stomach ache, lack of appetite, diarrhea and vomiting [41]. A serious threat to human health are also eggs of the nematodes *Toxocara canis* and *Toxocara cati* parasiting on carnivorous animals [42]. Humans are incidental hosts for these nematodes. The parasite does not develop in human body to the adult form, but remains in the internal organs at the L3 larval stage, causing toxocarosis [43,44]. According to the course of infection and location of larvae in the internal organs four clinical forms of the disease are distinguished: subclinical, visceral (organ), ocular and neurological.

The presence of parasitic worms in the gastrointestinal tract may exert an effect on the composition and functioning of the intestinal microbiota of the host, thus affecting modulation of the immune system and homeostasis of the whole body [45]. Studies on the effect of infection with parasitic worms on the intestinal microbiota in humans have been recently originated. Chronic invasions of intestinal parasites may affect human health by impairment of nutrition leading to malnutrition, vitamin deficiencies, micronutrient deficiencies (iron, zinc), decrease body resistance and effectiveness of prophylactic vaccinations [46–48]. They may also cause eosinophilic pneumonia [49] and anaemia [50]. Infections may be conducive to the occurrence of co-infections with other pathogens, exert an effect on their pathogenesis [51], and increase the risk of occurrence of allergic diseases [52].

The presence of invasive forms of intestinal parasites in the environment poses serious health risk for humans. Since 2009 there has been a lack of data concerning morbidity due to ascariosis, trichuriosis and toxocarosis among humans in Poland. This results from the fact of the removal of these disease entities from the Act of 5 December 2008 on the prevention and control of infections and infectious diseases in humans [53]. According to the registers by the National Institute of Public Health-National Institute of Hygiene (NIZP-PZH) kept in Polnd in the years 2003–2008 the number of cases of ascariosis was from 3,091–5,817 annually, that of toxocarosis – from 167–647 cases, whereas the number of cases of invasion with other gastrointestinal nematodes was from 9–23 [54]. In the subsequent years an upward tendency was observed in the number of reported cases of ascariasis and toxocarosis.

Apart from the register by the NIZP-PZH, few data concerning the degree of infection with gastrointestinal parasites in humans in Poland come from scientific studies and research projects pursued by scientific-research institutions and health care facilities [55]. Most frequently population screening studies for the occurrence of parasitic diseases of the gastrointestinal tract were carried out among children at school age, as a group at elevated risk of infection, as well as among participants of military operations, soldiers and their families, and hospitalized patients. A study by Wasilewska et al. conducted in 2011 [39] in eastern Poland showed invasions of *Ascaris* in 3% of 3-year-old children, 8.1% of children aged between 4–7, and in 15.8% of those aged 8–18. A study of a group of children and adolescents conducted by Żukiewicz et al. [56] demonstrated that the frequency of occurrence of ascariosis reached nearly 56%, while that of toxocarosis – more than 17%. Parasitological examination of faeces carried out by Raś-Noryńska et al. in 2011 [57] in a group of children and adolescents who did not show any symptoms directly related with invasion of parasites showed that 0.6% were infected with *Trichuris trichura* and 0.5% – with *Ascaris lumbricoides*. In addition, based on the result of a study conducted in the Łódź Province, a relationship was found between positive results of serologic tests for toxocarosis in children, and the presence of the eggs of *Toxocara* spp. in soil from patients' places of residence [58]. Indirect or direct contact with pigs infected with *Ascaris* spp. increases the risk of parasite invasion in humans performing work activities related with plant growing and livestock breeding [59]. To-date, no screening studies have been conducted in Poland for ascariosis, trichuriosis and toxocarosis in groups of people at a potentially elevated risk of infection. Considering the degree of contamination of soils with the eggs of geohelminths in Poland, it may be presumed that farmers may be a group exposed to parasitic diseases.

The general epidemiological situation of the occurrence of ascariosis, trichuriosis and toxocarosis in Poland is unknown. At present, no routine examinations are performed for parasite invasions among children at pre-school and school age, and generally a decrease is observed in the number of persons referred to parasitological stool examinations due to the suspicion of intestinal parasitosis.

In conclusions, the occurrence of invasive forms of intestinal parasites in soil may create a health risk for humans, especially those engaged in plant growing and livestock breeding. Based on the presented results of environmental studies concerning contamination of soil with the eggs of geohelminths *Ascaris* spp., *Trichuris* spp., *Toxocara* spp., and frequent contact with agriculturally used soil, it may be presumed that in Poland farmers and their families are the occupational group at an increased risk of invasion with intestinal parasites. Considering the degree of parasitic contamination of the soil environment and its participation in the food chain, also gardeners, forestry workers, fruit growers, persons engaged in livestock breeding, and growing vegetables and fruits for personal use may be exposed to the occurrence of parasitic diseases. The results of studies carried out in Poland indicated high a contamination of the soil environment in the rural areas with the eggs of intestinal parasites belonging mainly to the genera *Ascaris* spp. and *Toxocara* spp. Compared to the soil environment in urban agglomerations, in the rural areas considerably more sources of contamination of soil with invasive forms of geohelminths are observed. In order to protect soil against biological contamination, legal obligations are imposed on farmers associated with safe storage and use of solid and liquid fertilizers in agriculture. Despite the regulations in effect, the use of these fertilizers is not controlled. It may be presumed that an important factor which exerts an effect on the degree of the risk of infection is the adopted method of farm management, agricultural practices used, observance of sanitary-hygienic

principles, and the use of veterinary prevention.

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