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Contamination of vegetables, fruits and soil with geohelmints eggs on organic farms in Poland

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Abstract

The objective of this study was to evaluate the contamination of vegetables, fruits and soil with zoonotic parasite eggs on organic and conventional farms in south-eastern Poland. To evaluate the contamination with eggs of zoonotic parasites, examinations were conducted on 8 conventional and 11 organic farms in south-eastern Poland from May – October in 2008 and 2009. The following fruit and vegetables were selected for the experiment: strawberry, leek, onion, carrot, zucchini, beetroot, parsley, potatoes, celery, rhubarb, lettuce, cabbage, broccoli, pumpkin, young beetroot leaves, cauliflower, French beans, turnip, fennel and sorrel. A total of 187 samples of vegetables, fruits and soil were examined by means of a modified flotation method according to Quinn et al. (1980). Contamination with *Ascaris, Trichuris* and *Toxocara* eggs was found, with a higher number of positive samples revealed on conventional (34.7%), compared to organic farms (18.9%). The level of contamination in soil samples from conventional farms was higher (88.5% positive samples), than of those from organic farms (32.8%). Of the 15 geohelmints eggs, positive samples were found in vegetables: 9 *Toxocara* eggs, 4 *Ascaris* eggs and 2 *Trichuris* eggs. No geohelmints eggs were observed in the strawberry samples. The consumption of vegetables and fruits contaminated with the eggs of parasites may be the cause of parasitoses in humans. Stricter sanitary standards on farms of all types may limit the incidence of parasitic zoonoses.

Key words

vegetables, fruits, soil, parasites eggs, contamination

INTRODUCTION

Organic farming is the method of farming without using agrochemicals, synthetic mineral fertilizers, and chemical plant protection products. Before Poland joined the European Union (2004), the number of organic farms in the country was relatively small due to poor governmental financial support for this method of farming. Currently, the common agricultural policy provides financial support for organic farming, and thus provides incentives for Polish farmers to convert conventional farms into those of the organic type. In 2008, approximately 15,000 organic farms were registered, and organic crops cover 315,000 hectares, which accounts for 1.9% of arable land.

A great interest in organic farming is observed in the Lublin Region in south-eastern Poland, which is the area of the presented study. In 2008, there were 1,566 organic farms in this region, with crops of vegetables, fruits and cereals covering 30% of the cultivated land.

Both types of farms, conventional and organic, are subjected to sanitary control, and precise regulations cover the use of organic and organic-mineral fertilizers, specifying what types of fertilizers are appropriate for limiting biological contamination of soils.

The objective of this study was to evaluate the contamination of vegetables, fruits and soil with zoonotic parasite eggs on organic and conventional farms in south-eastern Poland.

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MATERIALS AND METHODS

Sampling. In 2008 and 2009, from May-October, examinations on conventional farms (CF) (n=8) and organic farms (OF) (n=11) in south-eastern Poland (Lublin Region) were carried out to evaluate the contamination of vegetables, fruits and soil with the eggs of *Ascaris*, *Trichuris* and *Toxocara*.

A total number of 187 samples from vegetables, fruits and soil were examined by the flotation method according to Quinn et al. [1]. Data to create a Table – 66 samples of vegetables, 8 samples of fruits, and 61 samples of soil from organic farms, and 23 samples of vegetables, 3 samples of fruits and 26 samples of soil from conventional farms. The list of the types of vegetables and fruits used for the study included: onion, zucchini, lettuce, leeks, celery, red beetroot, young red beetroot leaves, French beans, carrots, parsley, potatoes, broccoli, cauliflower, cabbage, turnips, fennel, pumpkins, rhubarb, sorrel and strawberries.

PROCESSING OF SAMPLES

Examination of vegetables and fruits. 2-3 individual vegetables of each type were picked from the soil, the excess of soil removed, collected in plastic bags, and brought to the laboratory. Strawberries were picked into the plastic containers – approximately 20 specimens. Vegetables and fruits were washed with 1 litre of tap water. After washing, the water was left for 24 hours for sedimentation. The liquid from the sediment was filtered through paper filters. After

staining the paper filters with Lugol's liquid iodine, they were examined under a microscope to detect parasite eggs. The sediment was examined by the modified flotation method [1]. The number of eggs was determined and differentiated with the use of the microscope.

Examination of soil samples. Soil samples were taken from the surface layer of soil, up to the depth of approximately 3 cm, at 5 sampling sites in the field, in the amount of about 0.5 kg each. A mean sample (from 5 component samples taken from each field) was designed for parasitological examinations. The soil samples were examined by means of the modified flotation method according to Quinn et al. [1]. A 100 g soil sample was flooded with 100 ml 0.0025% Tween 80, and subsequently shaken for 60 seconds. The suspension was filtered through flour mesh (pore size 180 μm) into centrifuge tubes and centrifuged for 10 minutes at 2,600g. Subsequently, the supernatant was poured off, 100ml 0.0025% Tween 80 solution was added again into the sediment, shaken and centrifuged for 10 minutes at 2,600g. After removing the supernatant, the sediment was flooded with 100 ml saturated NaCl solution, and centrifuged again for 10 minutes at 2,600g. After centrifugation, the tubes were supplemented with NaCl solution up to the convex meniscus and covered with a glass plate of the size 45×70 mm. After 30 minutes, the glass plate was carefully removed and observed under the microscope.

Statistical analysis was performed by means of Fisher's exact test.

RESULTS

The results of the presented study are given in Tables 1 and 2. The examinations showed a higher level of contamination with parasite eggs on conventional (26.9%), compared to organic farms (20.3%). However, statistical analysis did not show a significant difference between the results obtained on the 2 types of farms ($p>0.05$). The examinations did not revealed the presence of geohelmints eggs on fruits from either type of farm.

The higher number of samples were contaminated with the eggs of *Toxocara* spp. (CF – 19.2%, OF – 12.2%), followed by *Ascaris* spp. eggs (CF – 3.8%, OF – 5.4%) and *Trichuris* spp. eggs (CF – 3.8%, OF – 2.7%).

The results of presented study showed that the contamination of soil with parasite eggs was considerably higher on conventional than organic farms (88.5% and 32.8%, respectively). The highest difference in the percentage of positive samples on CF and OF was noted with respect

Table 1. Contamination of vegetables and fruits with parasite eggs

Species	Organic farms (No. of positive samples/No. of eggs)				Conventional farms (No. of positive samples/No. of eggs)			
	no. of samples	*Ascaris* spp.	*Trichuris* spp.	*Toxocara* spp.	no. of samples	*Ascaris* spp.	*Trichuris* spp.	*Toxocara* spp.
strawberry	8	-	-	-	3	-	-	-
leek	12	1/1	-	1/1	ND	ND	ND	ND
onion	7	1/1	-	2/2	1	-	-	-
carrot	7	-	-	1/1	4	-	-	3/3
lettuce	1	-	-	-	2	-	-	-
cabbage	2	-	-	-	3	-	-	-
turnip	1	-	-	-	1	-	-	-
potato	3	-	-	1/1	3	-	-	-
zucchini	7	-	-	1/1	ND	ND	ND	ND
broccoli	1	-	-	-	-	-	-	-
pumpkin	2	-	-	-	ND	ND	ND	ND
rhubarb	3	1/1	1/1	-	4	1/1	1/1	1/1
beetroot	6	1/1	1/1	-	4	1/1	1/1	1/1
young beetroot leaves	1	-	-	1/1	ND	ND	ND	ND
celery	4	-	-	1/1	1	-	-	-
parsley	5	-	-	-	3	-	-	-
cauliflower	1	-	-	-	ND	ND	ND	ND
French beans	1	-	-	-	1	-	-	-
fennel	1	-	-	-	1	-	-	-
sorrel	1	-	-	-	ND	ND	ND	ND
Total (%)	74	4 (5.4%)	2 (2.7%)	8 (10.8%)	26	2 (7.7%)	2 (7.7%)	5 (19.2%)
		14 (18.9%)				9 (34.7%)		

Table 2. Contamination of soil samples with parasite eggs

Species	Organic farms (number of positive samples/ number of eggs)				Conventional farms (number of positive samples/ number of eggs)			
	no. of samples	*Ascaris* spp.	*Trichuris* spp.	*Toxocara* spp.	no. of samples	*Ascaris* spp.	*Trichuris* spp.	*Toxocara* spp.
strawberry	12	-	-	6/13	3	-	-	1/1
leek	9	1/1	-	1/2	ND	ND	ND	ND
onion	6	1	1/1	2/2	1	-	-	-
carrot	3	-	-	1/1	4	-	-	3/3
lettuce	1	-	-	-	2	1/1	1/1	-
cabbage	1	-	-	-	3	1/1	1/5	1/1
turnip	1	-	-	-	1	-	1/3	1/ 2
potato	3	1/1	1/1	1/1	3	-	1/1	-
zucchini	7	-	-	-	ND	ND	ND	ND
broccoli	1	-	-	-	ND	ND	ND	ND
pumpkin	2	-	-	1/1	ND	ND	ND	ND
rhubarb	3	-	1/1	-	ND	ND	ND	ND
beetroot	3	-	2/2	-	4	1/2	3/53	2/9
young beetroot leaves	1	-	-	1/1	ND	ND	ND	ND
celery	2	-	-	-	1	-	1/1	-
parsley	1	-	-	-	3	2/3	1/14	1/ 2
cauliflower	1	-	-	-	ND	ND	ND	ND
French beans	3	-	-	-	ND	ND	ND	ND
fennel	1	-	-	-	1	-	-	-
Total (%)	61	2 (3.3%)	5 (8.2%)	13 (21.3%)	26	5 (19.2%)	9 (34.6%)	9 (34.6%)
		20 (32.8%)				23 (88.5%)		

to soil samples containing the eggs of *Trichuris* spp. (CF – 34.6%, OF – 8.2%) and *Ascaris* spp. (CF – 19.2%, OF – 3.32%). In both cases, the differences in the results obtained on farms of both types were highly significant statistically (*Trichuris* – p = 0.01, *Ascaris* – p=0.05). Also, on CF there were more samples contaminated with the eggs of *Toxocara* spp. (38.5%), compared to the results obtained on OF (21.3% of soil samples contained the eggs of this roundworm); however, the difference was not statistically significant. Statistical analysis did not show any significant difference between the numbers of the eggs of *Ascaris* spp. and *Toxocara* spp. in soil, fruits and vegetables from farms of both types (p>0.05).

DISCUSSION

The degree of contamination of the environment with the eggs of animal parasites is associated with the frequency of occurrence of animals in a given area, which are the source of parasites, also their age and level of resistance.

The source of the eggs of *Toxocara* spp. may be domestic animals (dogs and cats), and wild animals (foxes, wolves). In recent years, *Toxocara canis* was detected in 12.2% of stray dogs [2], in 6.4%-13.2% of country dogs [3, 4] and in 0.3%-2.7% of household dogs [3, 4]. The studies of helminthes infection in foxes and wolves from different parts of Poland showed the prevalence of *T. canis* infection from 19.1%-13.5%, respectively [5, 6]. The prevalence of *T. cati* in cats was 10.5%-24.2% in animal shelter cats, 26.1% in homeless cats, and 1.1% in domestic cats [7, 8]. The cause of the presence of *Trichuris* eggs in the environment is a high frequency of infection in animals (*Trichuris vulpis*, *Trichuris suis*) and in humans (*Trichuris trichiura*). In recent years in Poland, infestation of dogs and wolves with *Trichuris vulpis* was 6.8%-15.1% and 38.5%, respectively [6, 9]. Investigations carried out on pig farms showed infestation by *Trichuris suis* in 23.1% of piglets examined [10]. The prevalence of *Trichuris trichiura* infection in humans from post-infant until old age is several percent in Poland.

In Poland, the source of eggs of *Ascaris* are primarily swine, in which, according to age and breeding method, the infection with *A. suum* was recorded at 7.7%-61.5% [10]. Cases of ascariosis in humans are relatively frequently noted in Poland, and this mainly concerns children from the rural environment. Studies conducted by Wasilewska et al. in eastern Poland (impoverished areas of the country) showed infection of *Ascaris* in 3.0% of 3-year-old children, 8.1% of children aged 4-7, and 15.8% of children 8-18 years old [11].

Examinations of the contamination of the environment with the eggs of intestinal parasites are being carried out in urban and rural areas by researchers worldwide,. It is estimated that 0.3%-87% of soil worldwide is infected with the eggs of *Toxocara canis* [12]. In Poland, studies of contamination of soils most often concern such contamination with the eggs of *Toxocara* spp. In the area of eastern Poland, the eggs of *Toxocara* spp. were found in 37% of samples of soil from urban areas, in central Poland – in 27.1% of samples from urban areas, and 21,1% of samples from rural areas. In western Poland, 10%-20% of samples from the urban and rural areas contained eggs of *Toxocara* [13, 14, 15, 16].

Studies conducted in selected European countries confirmed the presence of the eggs of *Toxocara* spp. in a considerable percentage of soil samples. The highest soil contamination rates were observed in urban settlements, such as backyards (45% – Czech Republic, family gardens (38% – Ireland), parks (67% – Spain) and sandpits (87.1% – Germany), which were found to be heavily contaminated [17, 18, 19, 20]. A high level of contamination of the environment with the eggs of intestinal parasites (*Ascaris* spp., *Trichuris* spp. and *Toxocara* spp.) observed in many regions of the world, is associated with the high fertility of these geohelmints. A female of *A. lumbricoides* or *A. suum* lays about 200,000 eggs daily, while a female *T. canis* or *T. trichiura* – approximately 10,000 eggs [21]. In addition, eggs of geohelmints are very resistant to the effects of environmental factors. Eggs of *A. lumbricoides* may survive in the external environment and maintain their invasiveness for up to 6 years, whereas the eggs of *Toxocara* spp. even up to 10 years [22].

There are few studies pertaining to the infection of soil with parasites' eggs on Polish farms, especially organic farms. Kłapeć, while investigating the contamination of soil and vegetables on organic farms in south-eastern Poland (the Lublin region), found the presence of the eggs of *Ascaris* spp., *Trichuris* spp. and *Toxocara* spp. in 42.16% of soil samples, among which the samples containing the eggs of *Toxocara* spp. constituted 56% [23], similar to the above-mentioned studies. While examining soil from crops covered with foil shields on conventional farms in south-eastern Poland, the author observed contamination of soil with the eggs of *Ascaris* spp. and *Trichuris* spp. on the level of 25% for each parasite [24].

Studies concerning the contamination of vegetables, fruits, soils and irrigation water on conventional farms with the eggs of geohelmints have been conducted in many countries. For example, Erdogrul *et al.* in Kahramanmaras (Turkey) carried out studies of vegetables, fruits, soil and water used for watering for the presence of parasite eggs and cysts [25]. In all types of samples they observed the presence of the eggs of *Ascaris* spp., *Enterobius vermicularis*, and cysts of *Giardia* and *Entamoeba histolytica*. Other Turkish researchers compared the contamination with parasites of freshly washed and unwashed vegetables [26]. In 5.9% of unwashed vegetables they found the eggs of *Toxocara* spp., *Ascaris* spp. and *Taenia* spp. Daryani *et al.* indicated a similar relationship while investigating vegetables from gardens and markets in the area of Ardabil (Iran) [27]. Vegetables bought on the market (subjected to preliminary cleaning before sale) were contaminated to a lower degree than vegetables from urban household gardens, directly picked for the study (uncleaned).

The type of vegetables examined also exerted an effect on the results of studies because the level of contamination of leafy vegetables with the eggs and cysts of parasites observed was considerably higher, compared to root vegetables [28, 29]. The studies conducted by Gupta et al. showed that the factor which increases the contamination of vegetables with parasites may be untreated or incorrectly treated water used for watering crops [30]. As much as 83.3% of untreated water, and 68.2% of preliminary treated water contained the eggs of *A. lumbricoides*, *T. trichiura* and hookworms.

The presented examinations showed the presence of parasite eggs in vegetables and soil from both, conventional and organic farms, and confirmed that the degree of contamination of soil and vegetables on conventional farms was higher compared to organic farms. The reason of this

was probably due to the stricter system of standards in effect for the owners of organic farms, which has been recently introduced in Poland after it joined the European Union. Currently in Poland, Council Regulation (EC) No. 834/2007 is in effect, based on which organic farms are subjected to constant monitoring by certifying institutions, resulting in the improvement of the quality of plant and animal products, and the reduction in the level of contamination of soil and cultivated vegetables and fruits with the eggs and cysts of animal and human parasites. Authorized scientific institutes in Poland have the task of examining all organic and organic-mineral fertilizers applied in organic and conventional farming. The presence of invasive forms of parasites in the fertilizers examined makes it impossible to allow them to be used on farms. Also, animal breeding on organic farms is subject to constant veterinary supervision, which decreases the spread of parasites into the environment. Conventional farms, however, have no such limitations, and therefore, in the presented study, a considerably higher level of contamination of vegetables, fruits and soils on this type of farm was observed. Okulewicz et al. noted that in the rural areas nearly 100% of dogs and cats are infested with parasites [31].

Deworming of young dogs and cats does not entirely eliminate the problem, because some anti-worm drugs destroy only the adult forms of parasites, while the eggs preserve their capability for further development and may survive in the environment for several years. Thus, a considerable accumulation of the eggs in the external environment is noted [32]. A seasonal character of the occurrence of the eggs of *Toxocara* spp. in soil is also observed. Spring and summer are the times when young puppies and kittens are born and remain outdoors for a longer time.

The eggs of *Ascaris* spp. and *Trichuris* spp. found by the authors of the presented study in soil and vegetables examined were probably introduced with swine manure used as a fertilizer. They might also come from boars which frequently visited the areas of farms, as some of them were located in the vicinity of forests. Foxes, in which this parasite is frequently found, might also participate in the contamination of farms with the eggs of *Trichuris,*. In Poland, the population of foxes increased from over 50,000 in 1990 to 220,000 in 2006 [32]. A considerable effect on the increase in the abundance of foxes has been achieved annually by conducting actions of rabies control, initiated in 1995 in the regions of western Poland, and since 2002 carried out in the area of the whole Poland. In western Poland, *Trichuris vulpis* is noted in approximately 10% of foxes [33].

The examinations showed that parasitologically contaminated soil may be the source of contamination of cultivated vegetables. This contamination is observed on both organic and conventional farms. Vegetables contaminated with the eggs of parasitic roundworms may be the source of infestation of humans and animals, and create the risk of infection, not only of consumers, but also the hosts and holiday-makers visiting these farms.

CONCLUSIONS

The contamination of vegetables and soil with geohelminth eggs is much lower on organic than conventional farms. Food coming from organic farming should be considered as safe for consumers, provided that the basic principles of hygiene are observed, i.e. thorough washing of vegetables and fruits before consumption, and washing hands before meals. In both methods of farming the level of contamination of soil with the eggs of 3 types of roundworms – *Toxocara, Ascaris* and *Tichuris* – should be reduced by the deworming of dogs, cats, and breeding animals, as well as the fencing of farms located in the vicinity of forests inhabited by boars and foxes.

The consumption of raw vegetables and fruits contaminated with the eggs of *Toxocara* and *Ascaris* may be the cause of ascariasis and toxocariasis in humans. Toxocariasis is the most frequent zoonotic parasitosis in humans in Poland. Every year, solely in the area of central Poland, several dozen cases of toxocariasis in children are noted [32]. Therefore, the introduction of stricter sanitary standards on all types of farms, not only organic farms, may decrease human morbidity due to hooknoses in Poland.

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