Dynamics of *Echinococcus multilocularis* infection in red fox populations with high and low prevalence of this parasite in Poland (2007-2014)

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Abstract

The aim of the study was to estimate the current prevalence of *E. multilocularis* in selected populations of red foxes in Poland and to evaluate the changes in prevalence of this parasite by comparison with the results obtained in the same area during earlier surveillance. The investigations were performed in the area of four Polish provinces: 2 eastern/south-eastern (Lubelskie and Podkarpackie) and 2 south-western (Śląskie and Opolskie). Five hundred red foxes coming from the investigated areas were examined between 2013 and 2014 to estimate the current situation in selected provinces. Moreover, 550 red foxes from the same areas examined between 2007 and 2013 were used for comparison of differences in *E. multilocularis* prevalences in time. Intestines were examined with the use of the sedimentation and counting technique. Among 500 foxes examined in the current study, 118 were positive for *E. multilocularis*. There were differences in prevalence between individual provinces: Podkarpackie Province - 54.6%, Lubelskie Province - 18.9%, Śląskie Province - 11.7%, and Opolskie Province - 3.9%. Statistical analysis demonstrated that in most cases there were no differences in prevalence between the current results and the results from previous studies. Only in Opolskie Province was a statistically significant increase observed between 2010 and 2014. A stable degree of infection in the region with high prevalence of this parasite was demonstrated. However, a significant increase in the region with very low prevalence of *E. multilocularis* points out the necessity to monitor this infection during the coming years to control the progress of the disease.

Keywords: red fox, *Echinococcus multilocularis*, parasite prevalence, Poland.

Introduction

Human alveolar echinococcosis is a dangerous zoonosis caused by larval form of *Echinococcus multilocularis*. In most cases, the disease is fatal for untreated people. Humans play a role of non-specific intermediate host in the life cycle of this tapeworm; specific intermediate hosts are small mammals, generally arvicolid rodents and muskrats. Red fox (*Vulpes vulpes*) is the main typical final host of the parasite, which is responsible for spreading the infective eggs in the environment (9). Therefore, in many countries red foxes population surveillance was conducted to estimate the epidemiological situation. The knowledge about prevalence of this parasite in the main final host allows to conclude about infection risk in people. In Europe, in recent years, an increase in the prevalence of *E. multilocularis* in red foxes in endemic regions has been observed, and the expansion of this tapeworm to new areas has also been noted. Since the end of the 20th century, *E. multilocularis* has spread from a few countries regarded as the core region of this infection in Europe (Switzerland, Austria, southern Germany, and eastern France) (5), to several other countries, including Poland. It is undoubtedly connected with the real expansion of this parasite caused by many factors, among others, the increase in fox population. But on the other hand, this rise could probably be connected with an increased research interest in this parasite, and the introduction and
intensification of monitoring studies in the areas of Europe where they were not previously performed, or were conducted on a smaller scale. For example, in Sweden the first red fox infected with *E. multilocularis* was found in 2010, after ten years of surveillance carried out on a relatively low number of samples (about 300 per year) (22). Immediately after the first detection, an increased surveillance action was introduced, and after examining about 3000 foxes, the next 3 cases were found in 2011, during one year only (31). The first case of *E. multilocularis* infection in red fox in Poland was described in 1995 and it was connected with the beginning of a large monitoring action in the part of the country (18). Since this time, many investigations have been conducted in different regions of Poland and they showed diversity in prevalence of *E. multilocularis* in red foxes (1, 17, 23, 24). A study which was carried out recently in the whole territory of Poland confirmed non-homogeneous distribution of this infection (10). The rate of *E. multilocularis* infected foxes in some provinces reached 50% whilst in the other ones the percentage was low. The epidemiological situation in red foxes translates into the situation of human infections. In Poland, more than 120 cases of human alveolar echinococcosis have been recorded so far, and most of them coincided with the areas of high prevalence of the infection in foxes (20). The detection of *E. multilocularis* DNA in a large percentage of soil samples also indicates a significant contamination of the Polish environment in endemic areas (28). Moreover, in recent years in Poland larval forms of this parasite were found in pigs, which similarly to humans, play the role of non-specific intermediate host (11). Infection of pigs indicates the presence of infective eggs of this tapeworm close to human settlements.

Inferences about the risk of infection with this dangerous parasite for humans are still based on the estimation of epidemiological situation in red foxes – the main distributors of infective eggs. A recent Polish countrywide investigation (10) showed a significant increase of this infection in foxes in some regions compared to results obtained 20 years ago. This points out to the dynamic changes in the epidemiology of *E. multilocularis* in foxes. This tendency is continuously, regularly monitored – which is recommended by Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents. The possibility to make the analysis and compare it with the results obtained with the use of the same diagnostic method is especially important; however, it is not always possible when the comparison is made between the current and previous studies.

The aim of the study is to estimate the current prevalence of *E. multilocularis* in selected populations of red foxes in Poland and evaluate the changes in prevalence of this parasite by comparing the results with those obtained in the same area during the earlier surveillance.

Material and Methods

Study design. The investigations were performed in the area of 4 Polish provinces: 2 eastern/southeastern (Labelskie and Podkarpackie) and 2 southwestern (Śląskie and Opolskie) (Fig. 1). The red foxes (*Vulpes vulpes*) were hunted mainly as part of an official survey concerning the effectiveness of an anti-rabies vaccination. Only free-living animals were included in the study. The number of examined foxes was presented in Table 1. Five hundred red foxes from the investigated area were examined in 2013-2014 to estimate the current situation in selected provinces. Moreover, 550 red foxes from the same area examined in previous years (2007-2013) (10, 14) were used to compare the differences in *E. multilocularis* prevalence in time.

Small intestines were sampled and frozen for at least 7 d at -80°C before examination to inactivate the tapeworm eggs (7). The intestines were examined with the use of the sedimentation and counting technique (SCT) according to OIE Manual (21), adapted from the previously described method (8, 25).

Statistical analysis. Differences in the prevalence of the infection among the provinces in the current study and between the current and the previous results in the same province were investigated using chi-square test or V-square test (depending on the number of cases in groups). When multiple comparisons were made, Bonferroni correction was applied. Differences in the intensity of infection among the provinces in current study were estimated by Kruskal-Wallis test. Differences in the intensity between the current and the previous results obtained in the same province were analysed with the use of Mann-Whitney test. The differences in all analyses were considered statistically significant when P < 0.05. Statistical analyses were performed using IBM SPSS Statistics 20 and Statistica 9.1 Stat Soft.

Results

Among 500 foxes examined in the current study, 118 were positive for *E. multilocularis*. There were differences in parasite prevalence between individual provinces. The highest prevalence was noted in Podkarpackie Province (54.6%; 95% CI = 46.7 - 62.3) and the lowest in Opolskie Province (3.9%; 95% CI = 1.6 - 8.4) (Fig. 1). Statistical comparison between the provinces showed that the prevalence of *E. multilocularis* in Podkarpackie Province was significantly higher than in the other three provinces.
Fig. 1. Prevalence of *Echinococcus multilocularis* in red foxes in 4 provinces (gray colored on the map) of Poland (2013-2014): Opolskie (OP), Śląskie (SL), Lubelskie (LU), Podkarpackie (PK)

* - prevalences with differing letters (A,B,C) are significantly different (P < 0.05)

Table 1. Prevalence and intensity of *Echinococcus multilocularis* in red foxes in selected provinces of Poland - current results in comparison to previous studies

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Year of investigation</th>
<th>Number of examined foxes</th>
<th>Percentage of positive foxes (95% CI)</th>
<th>Mean number of worms per intestine (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Śląskie (SL)</td>
<td>2010&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102</td>
<td>11.8 (6.9 - 19.4)</td>
<td>513 (172%)</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>103</td>
<td>11.7 (6.7 - 19.4)</td>
<td>2923 (117%)</td>
</tr>
<tr>
<td>Opolskie (OP)</td>
<td>2010&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
<td>0.0 (0.0 - 3.7)*</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>155</td>
<td>3.9 (1.6 - 8.4)*</td>
<td>203 (217%)</td>
</tr>
<tr>
<td>Podkarpackie (PK)</td>
<td>2013&lt;sup&gt;c&lt;/sup&gt;</td>
<td>106</td>
<td>47.2 (37.9 - 56.6)</td>
<td>8704 (459%)</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>152</td>
<td>54.6 (46.7 - 62.3)</td>
<td>6135 (744%)</td>
</tr>
<tr>
<td>Lubelskie (LU)</td>
<td>2007-2008&lt;sup&gt;d&lt;/sup&gt;</td>
<td>242</td>
<td>18.2 (13.8 - 23.6)</td>
<td>519 (175%)</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>90</td>
<td>18.9 (12.0 - 28.3)</td>
<td>331 (200%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant differences between the current and the previous results in the same province (P < 0.05); <sup>b</sup> Karamon *et al.* (10), <sup>c</sup> Karamon *et al.* (14)

Moreover, its prevalence in Lubelskie Province was significantly higher than in Opolskie. There were no differences between the results obtained in Opolskie and Śląskie, and between Śląskie and Lubelskie.

The comparison of the percentages of the foxes infected with *E. multilocularis* in provinces obtained in the current study and in the previous investigation performed in the same areas is presented in Table 1. Statistical analysis showed that in most cases there were no differences in parasite prevalence between current and recent results. Only in Opolskie Province was a statistically significant difference observed between the results obtained in 2010 and 2014.

There were no statistically significant differences between the intensities of infection neither among the provinces in the years studied nor among the provinces in the current study.

**Discussion**

The distinct differences in parasite prevalence among the examined provinces confirmed the results of our previous investigation in Poland (10). This study showed an apparent division of Poland into two zones: “eastern half” with high prevalence of *E. multilocularis*, and “western half” with low prevalence of the parasite. In the current study 2 eastern provinces (Podkarpackie and Lubelskie) presented significantly higher prevalence of the parasite than
Opolskie Province located in the “western half”. In Śląskie Province, which is located between these zones, the prevalence is lower than in the eastern regions and higher than in Opolskie Province, but only in comparison with Podkarpackie Province is the difference significant. Another example of differences among some regions of one country located in endemic area is Slovakia, where distinctly higher prevalence of *E. multilocularis* was noted in northern provinces (Prešov, Žilina, Trenčín) than in the other parts of this country (19). However, in some countries this parasite is only noted in selected regions, *i.e.* in the Alps in northern Italy (2), northern part of Hungary (3), or northern Romania (26) – but in these cases the local occurrence of *E. multilocularis* is connected with the southern border of the distribution of this tapeworm across the continent.

In order to analyse the dynamics of *E. multilocularis* prevalence in 4 selected provinces of Poland, the results of the current and the previous investigations performed in the same areas were compared. The analysis indicated that during the recent years the situation did not change in the regions with very high and medium prevalence (Podkarpackie, Lubelskie, and Śląskie Provinces). The prevalence of the parasite in these provinces seems to be stable – the percentage of infected foxes was similar to the results obtained a few years ago. The comparison with the results obtained about 15-20 years ago showed that an increase in the parasite prevalence in these regions occurred much earlier – it began in the late 1990s and early 2000s (after the launch of the anti-rabies campaign). The prevalence level of *E. multilocularis* reached in this period has remained the same. However, a slight but statistically significant increase in the prevalence was observed in Opolskie Province. *E. multilocularis* was not detected in this province in 2010 (10), but in the current study (2014) about 4% of infected foxes were found. In this province, like in the rest of the “western half” of Poland, the sudden increase in *E. multilocularis* prevalence in foxes did not occur after the anti-rabies campaign at the turn of the 21st century. The currently reported results may indicate the beginning of an increase in the prevalence of the tapeworm in this region. This could only be confirmed just by an investigation carried out in the years to come. Such a delayed rise of the parasite prevalence in Poland was observed in Świętokrzyskie Province, which only occurred in 2008-2012 (from 3.6% to 17.5%) (10, 14).

It should be underlined that the analysis of the dynamics of infection presented in this paper was based on the comparison of the current and the previous results obtained by the same validation procedure (SCT) (12) and by the same research team, which guarantees its reliability. It is known that the methods used in echinococcosis diagnosis in foxes differ in effectiveness. For example, the intestinal scraping technique (IST), widely used in such surveys in the past, is characterised by significantly lower sensitivity and higher (worse) limit of detection (LOD) than SCT (8, 12, 13). This does not allow to compare precisely the results obtained with the use of these two different methods.

In general, the extension of *E. multilocularis* occurrence zone and the increase in the parasite prevalence in red foxes were observed in Europe during the last decades. In Germany, it was clearly indicated by a long-term investigation conducted in the same areas; *E. multilocularis* in foxes population increased from 12% to 42% between 1990 and 2009 in Central Germany (27), and from 12% to 20% in Northwest Germany during about 15 years (1991-2005) (6). Similarly, Combes *et al.* (4) compared their results (2005-2010) with earlier studies (1984-1987) and found a significant increase in *E. multilocularis* prevalence in foxes over time in most of the examined departments of eastern France. Moreover, mathematical calculation based on two examinations of red foxes between 1996 and 2003 in the Netherlands showed that the parasite prevalence is increasing in number and is spreading northward at the speed of 2.7 km per year (29). Furthermore, the expansive character of *E. multilocularis* infection was confirmed and described based on the analysis of the genetic diversity of this species in France (16, 30) and the whole Europe (15). In Europe, the increasing trend of *E. multilocularis* infection in red foxes is observed. Our investigation showed a stable level of infection in the regions with high prevalence of this parasite. However, a significant increase in the region with very low prevalence of *E. multilocularis* points out the necessity to monitor this infection during the coming years to control its progress.

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