Coproantigen prevalence of *Echinococcus* spp. in rural dogs from Northwestern Romania

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**Abstract.** In Romania, rural dogs have a significant role in transmission of zoonotic parasites, as they are in close contact with humans, but owners are less interested in controlling their free lifestyle and their food behavior. The use of modern immunodiagnostic techniques has improved the diagnosis of intestinal echinococcosis in our country and enhanced the possibility of making epidemiological studies on greater number of individuals. In this order, epidemiological data were collected, using the copro-ELISA technique for *Echinococcus* spp. coproantigens, from 1892 dogs which lived in rural areas of four counties from northwestern Romania (Bistrita-Nasaud, Cluj, Maramures and Satu-Mare). The overall coproantigen prevalence was 19.2% (364/1892), with the highest level of infection in Maramures county (34%), followed by Cluj county (28.3%) and Satu-Mare (12.2%). The lowest prevalence was registered in Maramures county (4.1%). It seems that the age of the dogs played a role in the level of infection with *Echinococcus* spp. (P<0.0001).

**Keywords:** Epidemiology; Intestinal echinococcosis; Dog; Rural area.

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**Introduction**

The use of modern immunodiagnostic techniques lead to the improvement of detection of *Echinococcus* spp. in definitive hosts (domestic and wild carnivores). A very good approach in this field is the detection of parasitic antigens from fecal samples (coproantigens) by the ELISA immunoenzymatic assay. This diagnostic technique has been used more often through the years, in large scale epidemiological screenings (Eckert et al., 2001), being also applied in Romania for the detection of dogs with intestinal echinococcosis (Seres et al., 2008).

Beside shepherd dogs from flocks of sheep, considered the main reservoir and risk of transmission of *Echinococcus* spp. to humans, it would be important to assess the evolution of this zoonotic parasite in dogs which live in rural environment, in order to evaluate the risk of human infection.
The main objective of this study was to obtain new epidemiological data on the rural dogs from northwestern Romania, concerning their parasitism with *Echinococcus* spp., using a more reliable diagnostic method, the copro-ELISA technique.

**Materials and methods**

The studies have been conducted during October 2005 – May 2008 at the Parasitology Department of the Faculty of Veterinary Medicine, Cluj-Napoca, on a number of 1,892 rural dogs from Cluj, Satu-Mare, Bistrița-Năsăud and Maramureș counties. The dogs were represented by males and females, and were grouped in four age categories as shown in table 1.

Fecal samples were collected, directly from rectum or from the ground. For safety reasons, the feces were stored at -20°C for at least 14 days before being further processed. The fecal samples were examined with the ELISA technique for the detection of coproantigens using the commercially available Chekit Echinotest (Bommeli, Liebefeld-Bern, Switzerland). The Chekit Echinotest is designed for the detection of *Echinococcus granulosus* and *E. multilocularis* coproantigens in dogs, foxes and cats, and is genus specific. The test was performed according to the manufacturer’s instructions, using 1 g of fecal material diluted 1:4 in the kit’s sample dilution buffer. The samples were run in duplicates. After centrifugation of the sample suspension (3,000 g at room temperature for 10 minutes), the supernatants were used for ELISA. Estimations of coproantigen level were carried out. The interpretation of results was made following the attached protocol from the above producer. Results were expressed in value %; values >40% were considered positive. The results were evaluated statistically using the GraphPad InStat software.

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Origin (County)</th>
<th>n positive</th>
<th>Prevalence (%)</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year</td>
<td>Males</td>
<td>Bistrița-Năsăud</td>
<td>160</td>
<td>34.0%</td>
<td>29.8%-38.5%</td>
</tr>
<tr>
<td>1-2 years</td>
<td></td>
<td>Cluj</td>
<td>137</td>
<td>28.3%</td>
<td>24.4%-32.6%</td>
</tr>
<tr>
<td>3-5 years</td>
<td>Females</td>
<td>Maramureș</td>
<td>24</td>
<td>4.1%</td>
<td>27.1%-61.1%</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td></td>
<td>Satu-Mare</td>
<td>43</td>
<td>12.2%</td>
<td>29.1%-16.2%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>364</td>
<td>19.2%</td>
<td>17.5%-21.2%</td>
</tr>
</tbody>
</table>

The prevalence of *Echinococcus* spp. coproantigens in dogs from Cluj county’s rural areas was 28.3%, with a higher level of infection in male dogs (30.5%) (table 3). Regarding age, the highest number of coproantigen positive cases for *Echinococcus* spp. was encountered in dogs aged between 3-5 years with a prevalence of 50.3% (table 3). The gender had no influence on the prevalence of *Echinococcus* spp. coproantigens in the studied dogs (p=0.086; Odds Ratio=1.36 with 95%CI=0.89-2.09; relative risk 1.08), but the age had influence on the sensitivity of dogs for the infection (p<0.0001). Prevalence of *Echinococcus* spp. coproantigens in rural dogs from the county of Bistrița-Năsăud, revealed by copro-ELISA method, was 34% (table 2), with the highest values in male dogs (36.4%) (table 3). Regarding age, the highest number of cases was registered in dogs aged 1-2 years with a prevalence of 47.6%.
Table 3. Prevalence (%) of *Echinococcus* spp. infection in rural dogs from Cluj (n=484), Bistriţa-Năsăud (n=470), Maramureş (n=586) and Satu-Mare (n=352) counties by gender and age

<table>
<thead>
<tr>
<th>County</th>
<th>Females</th>
<th>Males</th>
<th>&lt; 1 year</th>
<th>1-2 years</th>
<th>3-5 years</th>
<th>&gt;5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bistriţa-Năsăud</td>
<td>24.3</td>
<td>30.5</td>
<td>6.5</td>
<td>26.3</td>
<td>50.3</td>
<td>20.6</td>
</tr>
<tr>
<td>Cluj</td>
<td>28.8</td>
<td>36.4</td>
<td>0</td>
<td>47.6</td>
<td>33.8</td>
<td>41.7</td>
</tr>
<tr>
<td>Maramureş</td>
<td>2.6</td>
<td>4.8</td>
<td>0</td>
<td>2.4</td>
<td>7.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Satu-Mare</td>
<td>7.1</td>
<td>16.9</td>
<td>3.8</td>
<td>18.6</td>
<td>13.9</td>
<td>10.4</td>
</tr>
</tbody>
</table>

The gender had no influence on the prevalence of *Echinococcus* spp. coproantigens of dogs (p=0.064; Odds Ratio=1.41 with 95%CI=0.92-2.16; relative risk 1.12), but the age had influence on the sensitivity of dogs to infection (p<0.0001).

In rural areas from Maramureş county the prevalence of *Echinococcus* spp. coproantigens in dogs was low (4.1%) (table 2), the males being more infected (4.8%) (table 3). Regarding age, the highest number of cases was registered at dogs aged 3-5 years, with a prevalence of 7%. The gender had no influence on the prevalence of *Echinococcus* spp. coproantigens in dogs (P=0.15, Odds Ratio=1.86 with 95%CI=0.68-5.07; relative risk 1.02), but the age had influence on the sensitivity of dogs for contamination (P=0.034).

In Satu-Mare county the prevalence of *Echinococcus* spp. coproantigens in dogs from rural areas was high (12.2%) (table 2). Male dogs seemed to be more infected (16.9%) compared to females (table 3). Regarding age, the highest number of coproantigen positive cases was registered in dogs aged 1-2 years, with a prevalence of 18.6% (table 3). The gender and age had no influence on the prevalence of *Echinococcus* spp. coproantigens in dogs from Satu-Mare county (p=0.003; p=0.060 respectively).

Comparing the prevalence of *Echinococcus* spp. infection in rural dogs, from the four counties taken into study, we noted that in Bistriţa-Năsăud, it has been the highest percentage of coproantigen positivity (34%) (figure 1), followed by Cluj county (28.3%) and Satu-Mare county with 12.22%. The lowest prevalence was registered in the county of Maramureş (4%) (figure 1).

Considering the distribution of the positive cases, there was a significant correlation between the counties (P<0.0001), which justified their classification into areas with three types of endemicity: high endemic areas (Bistriţa-Năsăud and Cluj counties), medium endemic areas (Satu Mare county) and low endemic areas (Maramureş county).

**Discussion**

In our study, conducted in the four counties from northwestern Romania, we focused on dogs from rural areas, because although the infection is more likely to be present in stray and shepherd dogs, there is a risk of infection of humans from rural dogs also. The main problem is that parasite control treatments are not made regularly in villages, or there are used molecules with low broad spectrum anthelmintic efficacy, so that there are high levels of prevalence and intensity of cestodes in
this category of dogs. To this end, we wanted to determine the real level of infection with *Echinococcus granulosus* in this category of dogs, because their food is not always fully controlled by the owner (some dogs wander households), and the dogs can consume raw meat, possibly with cysts. In our study, significant differences were observed in the prevalence of *Echinococcus* spp. in rural dogs concerning their origin (county), which can be attributed to geo-local weather conditions, proper maintenance of the biological cycle of the parasite, interfering and other internal or external factors related to the individual and the possibility of contamination, and not least the education level of the population. In the studied dogs, the internal factors related to the gender didn’t play role in susceptibility to parasite contamination, but the age was involved in sensitivity, probably due to susceptibility to multiple infections increasing with age.

From the point of view of the impact on human contamination, it should be considered both the prevalence of parasite and the density of dogs which live around humans. In our research we observed that there was more than one dog in the same household, which come in daily contact with humans. Our results on the prevalence of infection with *Echinococcus* spp. in dogs in rural areas are significant because high values were recorded. This is a signal for the necessity to implement control programs in echinococcosis. These data are confirmed by other authors, that our country ranks among countries with high endemicity (Eckert et al., 2001). The percentage of positivity with *Echinococcus* spp. in dogs in rural areas (19.2%) of which a large part can be represented by *E. granulosus*, matches the data highlighted in the literature (Eckert et al., 2001). Thus, the author shows that prevalence of cestodes in urban dogs, particularly of *E. granulosus* is lower than in rural areas (range 50-62%). This may be due to weak infrastructure and poor public hygiene measures in rural environment, and the opportunities they have from many dogs in rural areas to contamination.

In our country, Iacobiciu et al. (2005) described a prevalence of 86.74% of human cystic echinococcosis and the average infection with *E. granulosus* in the definitive host in the years 1956-1997 was 15.4% in Romania. The detection of infection in dogs, in order to obtain real data on the prevalence of *Echinococcus* spp. in our country, has been difficult due to the use of diagnostic techniques with poor specificity and sensitivity and high risk of dissemination of eggs in the environment (necropsy of the small intestine, purgation with arecoline, identification of eggs by coprology). These shortcomings were overcome by introducing the use of ELISA technique for coproantigens and the epidemiological data became more conclusive because of the great number of dogs that can be examined in shorter time. We mention that this is the first epidemiological study performed on rural dogs and using the copro-ELISA assay in our country, in detecting the presence of *Echinococcus* spp.

Studies on the prevalence of intestinal echinococcosis in dogs from rural areas, were conducted in Western parts of our country only using purgation with arecoline and coprological examination (Morariu, 2004). In Timiș county, in Remetea village six dogs were examined, and only one (16.6%) was infected with *E. granulosus*. In Bucovat village, the prevalence of parasite ranged between 7.1% and 14.2%, while in Vărăș, it was between 11.1% and 44.4%.

Also in the Banat region, in two localities in Arad county, declared hyperendemic for human cystic echinococcosis, infection of dogs was on average 29.3%, mostly represented by stray dogs (47.6%) and shepherd dogs (38.9%). Guard dogs had 18.5%, followed by urban dogs who had lower levels of infection (6.2%) (Iacobiciu, 2001). In other countries, application of immunoenzymatic ELISA assay for early detection of *E. granulosus* coproantigens allowed obtaining more accurate data on prevalence in dogs (Craig et al., 1996). In a region of England, was performed an epidemiological study of the prevalence of echinococcosis in dogs, given the large number of cases of human cystic echinococcosis.

ELISA method was used to detect coproantigens and the prevalence was 22% (Allan and Craig, 2006), which does not differ
much from that obtained in our study. More recently, the detection of coproantigens is used to assess risk factors associated with infection (Moro et al., 2005).

In conclusion, the increased prevalence of parasitism with *E. granulosus* in dogs in the northwestern Romania shows poor concern about the control. Although there is a control program in this direction, it is partly and inconsistently applied. In addition, from the results it can be observed that the usual (three times a year) protocols of parasitic treatment don't take into consideration the prepatent period, therefore are insufficient for the control of this zoonotic parasite.

References
