ANCA MARIANA FLOREA

EPIDEMIOLOGY AND SEROLOGICAL DIAGNOSIS OF HUMAN CYSTIC ECHINOCOCCOSIS IN THE CENTRAL AND NORTH-WESTERN PART OF ROMANIA

(SUMMARY OF Ph.D. THESIS)

SCIENTIFIC COORDINATOR
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INTRODUCTION

Parasitism is the way of living in which a parasite lives compulsorily or optionally, temporarily or permanently on account of a host, from which it gets nourishment, using either the nutrients or the metabolic products of its holder. *E. granulosus* species determines diseases that may be included in the large group of parasitic zoonoses, namely the diseases transmitted naturally from animals to humans and vice-versa.

Among the multiple diseases determined by parasites, cystic echinococcosis belongs particularly to the parasitosis which has the most severe and varied implications both in pathology and in public health issues. Considered as a “disease of human negligence and lack of education” (Bouree, 2001), this zoonosis stands on the first place, together with the trichinosis, among the zoonosis of Romania.

REASON AND PURPOSES OF THE SCIENTIFIC RESEARCH

Even if we have much information about the hydatid infestation, this disease is still wide spread both in humans and in animals. Practically it is spread in the whole world, and there are a few and restricted surface neutral areas. Taking into consideration the high costs of paraclinical diagnosis methods, of hospitalization, of surgical and medicinal treatment, the temporary work incapacity and the days of medical leave, the psychosocial accommodation, the possible recurrences and, as a last consequence, the possible morbidity, it is necessary to prevent the apparition of the disease, fact that implies a long-time investment in order to stop its transmission. On the other hand, due to the fact that the parasite develops in the body of domestic animals, this parasitic disease also determines an important economic loss.

Twenty years ago, Coroiu (1998) carried out studies regarding the incidence of the human cystic econococcosis in the north-western and central part of Romania. The endemic situation presented by the author in that period, the fact that in this area people use to breed and slighter animals in their own farms and the transhumance determined us
to consider that is necessary to know the spreading of the parasite and its evolution in this part of the country and in this moment.

In this work we have intended to fulfill the following purposes:

- To identify the real incidence of the hydatid cyst in Cluj county (situated in the north-western and central part of Romania), by carrying out a retrospective study on surgical cases;
- To follow the evolution of the hydatid disease in the whole Cluj county and in relation with the categories of population taken into consideration in the study;
- An evaluation of the level of infestation with the larval form of *E. granulosus* parasite in the human population of the north-western and central part of the country, by prospective investigations regarding the presence of the anti-*Echinococcus* antibodies;
- To determine the categories of population exposed to a higher risk of infestation;
- To draw hydatid disease prognoses, in the whole area, depending on sub-areas and categories of population;
- A serological evaluation, using the ELSA technique, in the cases hospitalized in the Surgical Clinic no. III of Cluj-Napoca, underling the role of the immunodiagnostics in the correct detection of the disease and in the prophylactic and therapeutic control of cystic echinococcosis in humans;
- To correlate the serological results with those obtained from other investigation methods.

Further on there is a brief presentation of our targets, mentioning the purpose, the materials and the working methods, the results and the discussions for each target.
1. FREQUENCY, DISTRIBUTION OF *E. GRANULOSUS* METACESTODE IN THE HUMAN POPULATION OF THE STUDIED AREA

Hydatidosis or cystic echinococcosis is an infestation caused by the larval form of *E. granulosus* species. It may develop asymptptomatically or as a severe disease, often fatal, in humans (Eckert, 2002). Cystic echinococcosis is a zoonosis that affects Romania too, our country being situated at the border line of the large international area in which human cystic echinococcosis is endemic, namely the basin of the Mediterranean Sea.

1.1. INCIDENCE, DISTRIBUTION AND TENDENCY ON THE BASIS OF THE SURGICAL CASES

**Purpose**

In this study we have intended to follow the evolution of the human hydatid disease in time, in a 10-year period (2000-2009), to characterize its distribution depending on different categories of population and on the area of Cluj county.

**Material and methods**

- The surgical cases of Cluj county, from 2000-2009, taking into consideration only the cases of first-infestation. The individuals who had more hospitalizations in the same hospital or in different hospitals were taken into consideration only once, when the disease was discovered. We selected the information from the admission records, the surgery protocols and the medical charts. The information we have taken from these documents referred to: name, gender, age, residence at the level of the village, of the parish, of the city, of the county, occupation, admission and release diagnosis;

- We calculated the multiannual average morbidity rates and the disease evolution tendency (the trend line) in general population and depending on categories of population (urban/rural, females/males, adults/children);
• We applied the statistical $\chi^2$ test with Yate’s correction.

**Results and discussions**

In Cluj county, during 2000-2009, we pointed out 323 cases of hydatid cyst, which underwent surgical intervention. In these cases the presence of the larval invasive stage of the parasite determined the apparition of some major disease symptoms. WHO recommends using the information of the surgical services in order to measure the incidence of the parasite in the human population, even if they reflect only a part of the parasitic cases (Eckert et al., 1995).

The cases come, in a higher percentage, from the rural environment (52,60) and among females (52,30). Regarding the proportional distribution of the hydatosis on age groups we noted an increase of the percentage on successive ages, both in adults and in children, with higher levels in the groups of ages which were more exposed to the infestation possibilities for a longer period. In adults the range of ages which was more affected by the disease is between 20-59 years, resulting a percentage of 69,1 cases.

In order to estimate more exactly what these cases represent for the population, and to determine the dispersion of the parasite among the population, we calculated the morbidity rate per 100,000 inhabitants. The average morbidity rate in Cluj county in a 10-year period is 4,6 cases per 100,000 inhabitants (fig. 1). This value reflects the geographical area in which we are. It is situated between the low parasitic level of the northern Europe, which is less affected by this zoonosis, and the hyperendemic southern Europe, where morbidity rates often exceed the value of 10 cases per 100,000 inhabitants (Eckert et al., 2002).
Figure 1. Average multiannual incidence of human cystic echinococcosis in Cluj county

Incidence of human cystic echinococcosis in urban and rural, in females and males, in adults and children, over 10 year period in Cluj county

Table 1.

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</table>
If we compare the multiannual average morbidity rates in the urban environment—3,3\%/oooo and in the rural environment—7,4\%/oooo, we find out obvious differences between the frequency of the parasite in the two environments ($\chi^2=61,7$; 
$p<0,01$) (figure 1). Our data regarding the particular affectation of the rural population underlines the fact that the highest incidence is given by the rural population (Romig et al., 2006).

Depending on genders, the multiannual average rate is nearly similar, slightly higher in females—4,7\%/oooo than in males—4,6\%/oooo ($\chi^2=6,1$; $p=0,01$) (figure 1). Many statistics mention a higher frequency of the parasitosis in males, but they consider that females represent a category which is more exposed to the risk of infestation (WHO, 2003).

If we compare the morbidity rates in adults and children we note that these are higher in adults—5,1\%/oooo than in children—2,9\%/oooo, in the studied period (figure 1). Most of the authors consider that the maximum frequency of the parasite is at the active age, between 20 and 60 years, when humans may be easier infested due to their activities (Lupașcu and Panaitescu, 1968). The lower incidence of the disease in children must not be neglected, since children, due to their specific activities, represent the most exposed group, with the most recent rate of transmission of the parasite (Abu-Hasan et al., 2002).

In order to characterize the parasitic phenomena in time, we followed the incidence of the parasite in the population depending on years (table 1). We found out variations of the annual rates between the minimum of 2,3\%/oooo in 2009 and the maximum of 6,2\%/oooo in 2005 (table 1). In order to eliminate these variations and to find out the real evolution in time of *E. granulosus* parasitism in the studied area, we calculated and drew the trend line. It was situated in obvious decline. The descendant evolution of the parasitosis during 2000-2009 reflects the social-economic conditions of the area: aging of the population of villages, modification of the working style in the rural environment, decrease of the sheep flocks, etc.

Following the evolution of the parasitosis in Cluj county may underline some modifications in its distribution at the same time with the social-economic changes of the area. We know the economic crisis that emerged at the end of 2009, crisis which determines, due to the increase of the unemployment rate, of the lack of jobs and of the poverty, a migration of the population from the urban to the rural environment, where
most of the people begin to practice again the agricultural activities. Precisely due to these considerations, we have considered that it is necessary to draw the tendency line for a 28-years period (1981-2009). It was slightly ascendant, which is a proof of the active transmission of the parasite.

If we compare the morbidity rates depending on origins and years, we note that during all these years the rates are higher in the rural environment (except 2009), even if the distribution of the parasite in this environment has variations from one year to another. We have noted oscillations of the annual values in the rural environment between the minimum of 2.20/oooo in 2009 and the maximum of almost 10o/oooo in 2005 and 2008 or even higher than 10o/oooo in 2000 and 2004 (table 1). The culminating points of these years prove an intensification of the causes that determine the difference between the two environments. The evolution tendency of the hydatid disease during the 10 years we have studied, depending on environments, shows decreasing prognoses of the parasitosis frequency in the rural environment in comparison with the urban environment, where the tendency is linear. The decrease of this trend in the population of Cluj county involves the decrease of the disease trend in the rural environment too, taking into consideration the fact that the rural population is unquestionably more exposed to the risk of parasitation due to the agricultural activities, to the animal breeding in their own farms, to the lack of sanitary education, etc.

The evolution in time of the disease depending on genders shows a great variability of *E. granulosus* infestation in successive years, prevailing one gender or another (table 1). It is difficult to give appreciations regarding this distribution, which may depend on different factors involved in the epidemic process or may be accidental (Todorov and Boeva, 1999). The descendant evolution of the parasitosis both in females and in males shows the fact that the disease manifests itself in the same direction and degree in both genders. Yet, if we calculated and drew the trend line for a 28-years period (1981-2009) we noted that females represent the group of risk in Cluj county, while males represent the category which is affected by the disease.

If we calculate the multiannual incidence in adults and children we note the presence of the *E. granulosus* parasite in both categories during the whole years of study (table 1). The evolution of the disease is descendant both in adults and in children, the
trend line having higher amplitude in adults due to the higher morbidity rates of the whole years. Yet, the presence of the parasite in children explains the recent rate of transmission of the parasite, unlike its presence in adults, fact that explains the tardive cases of the disease.

The basic reproductive report—marked Ro—is fundamental in order to understand the dynamics of *E. granulosus* parasite populations. The value of Ro characterizes the state of the parasite population. It may have the following values:
- *Ro>*1 characterizes the endemic state,
- *Ro>*1 characterizes the hyperendemic state,
- *Ro<*1 characterizes the extinction state,
- *Ro=*1 characterizes the survival state.

We followed the dynamics of the parasite transmission in the whole Cluj county, by calculating the morbidity rates depending on ages, using the surgical cases of 2000-2009 (table 2).

| Age groups | 0-4 | 5-9 | 10-14 | 15-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 | 65-69 | 70 | Total |
|------------|-----|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|    |-------|
| Cases no.  | 0   | 9   | 11    | 23    | 24    | 20    | 19    | 20    | 18    | 23    | 37    | 30    | 29    | 25    | 35 | 323 |
| Incidence o/0000 | 0  | 2.5 | 3.0   | 4.6   | 4.5   | 3.5   | 3.4   | 3.2   | 4.6   | 5.1   | 7.4   | 6.8   | 9.1   | 7.6   | 5.1 | 4.6 |
| Males cases no. | 0  | 5   | 5     | 9     | 15    | 11    | 11    | 7     | 10    | 7     | 13    | 18    | 10    | 12    | 21 | 154 |
| Incidence o/0000 | 0  | 2.7 | 2.6   | 3.4   | 7.4   | 3.8   | 3.9   | 2.2   | 5.1   | 3.1   | 5.4   | 8.8   | 6.8   | 8.3   | 6.6 | 4.6 |
| Females cases no. | 0  | 4   | 6     | 14    | 9     | 9     | 8     | 13    | 8     | 16    | 24    | 12    | 19    | 13    | 14 | 169 |
| Incidence o/0000 | 0  | 2.3 | 3.3   | 6     | 2.7   | 3.2   | 2.9   | 4.2   | 4.1   | 6.9   | 9.3   | 5.1   | 11    | 7.1   | 3.8 | 4.7 |

The multiannual average morbidity by hydatid cyst in Cluj county has increasing values for the age group of 15-19 years, but after that there are variations from one group to another. In order to eliminate the oscillations that appear from one group to another and to estimate objectively the dynamics of the parasite transmission in the human population of Cluj county we have calculated and drawn the trend line. The ascendant tendency of evolution in the whole age groups shows the fact that infestation was
produced in the human population, on the contrary from what resulted from the tendency of evolution of the hydatid disease in adults and children, where we have noted an amelioration of the disease prognosis. In the actual case $R_o > 1$, which means that in Cluj county *E. granulosus* parasitism is characterized by endemcity.

If we calculate the annual morbidity on genders and age groups we may note an increase of the hydatid cyst morbidity in females and males, but in different range of ages. Thus, males reach the maximum level at the age of 55-59 years, when the incidence rate is 8,80/oooo, and the maximum level reached by females is at the age of 60-64 years, with the incidence rate of 11o/oooo (table 2). If we follow the parasitation intensity curve/age and draw the trend line in both genders we note the fact that these are highly ascendant and perfectly overlapped, which proves that the infestation was produced in the similar way both in females and in males. In the actual case $R_o > 1$ both in females and in males, which means that *E. granulosus* parasitism is characterized by endemcity.

If we analyze the regional distribution of the human cystic echinococcosis in Cluj county during 2000-2009, we note the existence of neutral areas in which there were not cases of operated hydatid cyst for 10 years. There are 25 villages of the total of 75 villages of Cluj county in this situation, representing 33,3%. In 32% of the county the multiannual average morbidity surpasses 5 cases per 100,000 inhabitants and in 27% of the cases it surpasses 10 cases per 100,000 inhabitants. In the areas in which the multiannual average morbidity by hydatid cyst surpasses 10 cases per 100,000 inhabitants, it is necessary to implement surveillance and control programs of this parasite, whose impact on the health of the human population cannot and must not be neglected.

A review of the larval form localization in the human body of the 323 surgical cases shows its hepatic preponderance, followed by the pulmonary preponderance. Liver was affected in 257 cases, fact that represents a percentage of 79,6. The localization of the parasite at the pulmonary level, in unassociated infestation, appeared in 52 cases, which means a percentage of 16,1. After the hepatic and pulmonary localization, there are multiple localizations, with a percentage of 1,2, the value of the other localizations being reduced in Cluj county during 2000-2009. A comparative study of the main localizations of the hydatid cyst in children and adults shows a higher percentage of pulmonary
localization in children (32,6) than in adults (13,6). The statistical estimations ($\chi^2=69,1; p<0,01$) show that there are significant differences between the pulmonary localization of the hydatid cyst in children and in adults, which are not due to chance but to some objective causes, that are not completely elucidated at the present.

In Cluj county, during 2000-2009, the highly infestation risk occupations are in the rural environment (51,1%), which is a proof that human cystic echinococcosis is still a “first emergency disease of farmers”, Deutz et al., (2000). It is worth to mention that there are enough cases (48,9%) of people who had no connection with the rural environment or with occupations that support parasitary infection. Here we may speak about people’s habits and negligence.

**Conclusions**

1. Due to the multiannual average morbidity of 4,6 cases per 100,000 inhabitants, Cluj county is situated among the endemic areas;
2. The dynamics of the social-economic conditions determines modifications of the infestation risk, which are underlined by the tendency of evolution of the disease;
3. During 2000-2009 the prognosis of the hydatid disease is favorable, but if we take into consideration the double epidemiological potential of the disease, due its coexistence in natural and synanthropic focuses, the surveillance of the evolution of the parasitosis in humans must not be abandoned;
4. The presence of the hydatid cyst at the age group of 5-9 years proves the recent nature of the infestation;
5. The presence of multiple localizations and of other visceral localizations besides the liver and the lungs, is a proof of endemicity;
6. The high frequency of the parasitosis in individuals who apparently had no connection with the rural environment or with occupations that support the parasitary infestation, explains the constant maintenance of the parasite in the county and, implicitly, of the endemicity.

**Purpose**

The serological tests are important not only for the diagnostication of the hospitalized cases, but also for knowing the distribution of the parasite in different areas, in order to implement surveillance and control programs of the disease determined by this parasite.

This study plans to estimate the level of distribution of the parasite in apparently healthy population by establishing the group prevalence of the anti-\textit{Echinococcus} antibodies.

**Material and methods**

- We carried out a serological screening in 683 individuals without disease symptoms, establishing the group prevalence in samples of six counties of the central and north-western part of the country: Bihor (92), Satu-Mare (119), Salaj (141), Bistrita-Nasaud (84), Cluj (138) and Alba (109);
- We used ELISA immunoenzymatic tests for IgG antibodies, and worked with German NovaTec kits. The results were expressed in NovaTec kit units (NTU). The values below 9 NTU are considered negative, those between 9 and 11 NTU are not convincing and those above 11 NTU are positive;
- We analyzed the distribution of the anti-\textit{Echinococcus} antibodies depending on the origins of the seropositive individuals, on sexes and age categories, in order to establish the hydatid infestation risk groups;
- We realized a prediction of the hydatid disease by expressing the positive predictive value, and the calculation formula was based on Bayes’ theorem of conditional probability;
- We applied the “Student” statistical test (“t” test).
Results and discussions

Among the 683 tested serums, 35 were reactive in the ELISA test, resulting a general seropositivity of 5.12%. Depending on the categories of population, on the whole investigated area we noted a higher level of seropositivity in the rural environment (7.24%), than in the urban environment (2.77%), in females (6.72%) than in males (3.55%) and similar values, slightly higher in adults (5.15%) than in children (5.05%) (figure 2).

![Figure 2. Global level of the serological tests](image)

The frequency of the hydatid antibodies in humans is not similar to the presence of the hydatid cyst, even at asymptomatic levels (Barbieri et al., 1994; Arda et al., 2009). We consider that even in the case of the investigated area the frequency of the antihydatid antibodies in humans will not show actually the presence of the hydatid disease. In order to give a prediction of the hydatidosis in apparently healthy population, we applied the calculation of the positive predictive value on the serological results. The positive predictive value of 50.6% shows that the positive serological results correspond to the presence of the hydatid disease in the bodies in the same percentage. If we apply the positive predictive value at the level of the general seropositivity we have obtained, it becomes 2.59% instead of 5.12%, a level where we predict the presence of the hydatidosis in the apparently healthy population of the investigated area (figure 2).
We may note variations of the seropositivity and implicitly of the predicted disease from one area to another. The prediction of the hydatid disease is situated between the minimum of 1.96% in Salaj county, 2.02% in Cluj county and the maximum of 3.19% in Satu-Mare county and 4.63% in Bihor county (table 3). In the asymptomatic population of the investigated area there are gradations of the level of *E. granulosus* parasitation, gradations which are due to the interactions between the host parasite and the environment, but also to the characteristics of each one. Even with these constant variations, the hydatid antibodies were present in the investigated area. It proves the presence of the parasite and the existence of the perpetuation conditions of the biological cycle, with direct consequences on its transmission to the humans.

Global results of the serological tests

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</tbody>
</table>

The frequency of the hydatid antibodies and of the asymptomatic predicted disease is higher in the rural environment in the whole counties we have studied. The risk category belongs to the rural environment. In the whole area we may note a frequency of antibodies of 7.24% in the rural population and of 2.77% in the urban population (figure 2). The difference is significant (tcalculated=267, p<0.01). The probability of catching this parasitosis is significantly higher in the rural environment. It is obvious that the infestation of the environment with the eggs of the parasite, eliminated through dog excrements, is much more accentuated in the rural than in the urban environment. This situation is maintained due to the fact that people do not know the way of transmission of the parasite and due to the human behavior when feeding dogs and to the elimination of the infested organs resulted from slaughtered animals. At the level of the counties we may note variations of the R/U report regarding the level of the antibodies in the
population and the probability of the existence of non-manifestative parasitosis. The report of the predicted disease is lower in Alba (R/U=2.4) and Bihor (R/U=2.2) counties and very high in Salaj (R/U=8.9) and Cluj (R/U=18.2) counties (table 3). The variations of the R/U report regarding the prediction disease appear especially due to the dynamics of the social-economic factors of these counties and to the maintenance of a permanent relation between the two environments.

The distribution of the antidydatid antibodies on genders shows a higher level in females (6.72%) than in males (3.55%) (figure 2). The difference between the antidydatid antibodies in the two genders is significant from the statistical point of view, for a tcalculated=189.78, which corresponds to an error threshold p<0.01. On the basis of the statistical calculations we consider that the females are a category much more exposed to the risk of infestation. In the whole area the prediction of non-manifestative hydatid disease is 3.88% in females and 1.46% in males, with a F/M report of 2.58 (figure 2). In the whole areas of the investigated territory, both the level of the antibodies and of the predicted disease are higher in females, but with important variations from one county to another. The most significant differences are in Satu Mare county (F/M=6.7) and in Bihor county (F/M=4.9). The differences of the prediction disease are lower in the population of Cluj county (F/M=1.3), and Salaj county (F/M=1.4) and Bistrita-Nasaud county (F/M=1.4), but both antihydatid antibodies and hydatid disease prediction prevail in females (table 3).

These results show the fact that the infestation risk, which is related to the people’s direct or indirect exposure to dog excrements, due to local and occupational conditions, has variations in this area. This situation is also due to people’s habits, and this fact should be taken into consideration when they will implement surveillance and control programs of this parasitosis.

If we compare the hydatid anti-Echinococcus in the adult and the infantile population we may note that their level is close, slightly higher in adults than in children. In the whole area we may note a 2.61% frequency of antihydatid antibodies in the adult population and 2.52% in the infantile population, without significant statistical differences between the two categories (figure 2). In Bihor and Salaj counties there is no hydatid disease prediction, but in Satu Mare county the hydatid disease prediction is
higher in children (5.95%) than in adults (1.21%). In the other areas, the predicted values are relatively close (table 3), but the presence of the antibodies in the infantile population, even at a smaller level, shows the most recent rate of transmission of the parasite and the proof of its maintenance in the area, and the endemic character of the hydatid disease.

Conclusions

1. We predict the presence of hydatosis of 2.59% in the population of the investigated area.
2. The presence of the anti-\textit{Echinococcus} antibodies in the whole area, but with different intensity of parasitation from one area to another.
3. Depending on the categories of population, we may note a significantly higher level of the seropositivity in the rural environment (7.24%) than in the urban environment (2.77%), in females (6.72%) than in males (3.55%) and close values, slightly higher in adults (5.15%) than in children (5.05%), in the whole investigated area.
4. The presence of the hydatid antibodies in the infantile population shows the most recent rate of transmission of the parasite, a proof of its active maintenance in the area.


It is known that we need highly specialized personnel for a surgical intervention in the case of cystic echinococcosis with different localizations (liver, lungs, kidneys, brain, spleen, etc). The Surgical Clinic no. III of Cluj-Napoca, which is famous for the surgical interventions specialized in the digestive system, is the clinic with the most surgical interventions of hydatid cyst of Cluj county. Taking into consideration the fact that human cystic echinococcosis is more frequently localized in the liver we choose this clinic to identify the whole cases hospitalized here.

**Purpose**

On the basis of the surgical cases we have carried out an epidemiological study of the sample of patients hospitalized for hydatid cyst in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009. Thus, we have recorded the whole cases of hospitalized and operated hydatid cyst in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009. We have analyzed the operated cases depending on their origins, on sexes, on age groups and on their occupations. At the same time, we have studied the localizations of the hydatid in the body and the postoperative complications that may appear. Knowing the fact that human cystic is a disease with recidive tendency, this study allows us to identify the individuals who needed more hospitalizations in this clinic.

**Materials and methods**

- The information about the patients was processed from the hospital documents: admission records, operatory protocols and patients’ charts. We have followed attentively the hospitalization period, the days of hospitalization, the certain diagnostics, the localization of the cyst in the body, the postoperative complications. At the same time, we have studied the anamnesis, the patients’ history of the disease, the paraclinical and the laboratory investigations.
- We have applied the “Student” statistical test (“t”).

**Results and discussions**

185 individuals (table 4) were hospitalized and operated of hydatid cyst in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009. The number of the cases does not
certify the reality, because the operated cases represent only the top of the iceberg in the case of the disease.

Distribution of human cystic echinococcosis cases operated during 2006-2009, in the Surgical Clinic III, Cluj-Napoca

<table>
<thead>
<tr>
<th>Year</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>59</td>
<td>39</td>
<td>55</td>
<td>32</td>
<td>185</td>
</tr>
</tbody>
</table>

The investigated patients came from 20 counties, including Cluj county, most of the counties from the central and north-western part of Romania. Most of the cases of operated hydatid cyst belong to Cluj county (24,9%), followed by Maramures county (14,6%), Alba county (11,4%), Hunedoara (9,7%), Bistrita-Nasaud (9,2%), Salaj (7,6%), Bihor (4,4%), Sibiu (3,2%) and Satu Mare (3,2%).

The 185 cases come from 118 localities. We discovered more cases of operated hydatid cyst in some localities. There are 14 localities of the rural environment (11,9%) in this situation.

50,8% of the 185 cases of cystic echinococcosis hospitalized in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009, came from the urban environment, and the rest of 49,2% came from the rural environment. By the statistical estimation we have found out that there is no statistical significance in the way of presentation of the individuals belonging to the two environments when they came to hospitalize (tcalculated=0,1 for p>0,01). The results we have obtained may be explained by:

- The maintenance of permanent relations between the two environments;
- The high number of people affected by this parasitosis belonging to the urban outlying areas, due to misery and poverty;
- The high number of homeless dogs;
- Higher accessibility and addressability to specialized institutions in the urban environment than in the rural one;
- The early discovery of the cases in the urban environment, due to periodical examinations at the place of work.
54.6% of the 185 cases of cystic echinococcosis hospitalized in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009, were females, and the rest of 45.4% were males. By the statistical estimation we have found out that there is no statistical significance in the way of presentation of the individuals belonging to the two categories when they came to hospitalize (t_calculated=0.5 for \( p>0.01 \)). The fact that females come in higher number than males may be explained by:

- Direct or indirect exposure of females to dog excrements due to their living conditions and to their lifestyle;
- Females’ higher sensibility and vulnerability to the diseases than males’.

Regarding the distribution of cystic echinococcosis cases hospitalized in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009, on age groups, we have noted that the parasite is present beginning with the age group of 0-14 years till 70 years old. There is an increase of the affected percentage on successive age groups, from 8.1 in the age group of 0-19 years, till 43.1 in the age group of 40-59 years, after that the percentage decreases at 24.3 in 60 years old individuals. The lowest frequency of the infantile hospitalized cases (8.1%) is explained by the fact that the majority of the children are hospitalized in the Infantile Surgical Clinic of Cluj-Napoca, due to the fact that the Surgical Clinic no. III is specialized in adults.

In our study, the predominant localization of the hydatid in the hospitalized cases is the liver, followed by the abdomen, taking into consideration the fact that the Surgical Clinic no. III is specialized in digestive diseases. The percentage of hepatic localization was 90.3, followed by multiple localizations of 5.4 and peritoneal localization of 1.6.

The double localizations were:

- Hepato-peritoneal (4 cases)
- Hepato-renal (2 cases)
- Hepato-pancreatic (1 case)
- Pancreatic-epiploic (1 case).

The triple localizations were:

- Pelvic-subperitoneal-epiploic (1 case)
- Spleno-intravesical-uterine (1 case).
The affectation of multiple organs, in the same host, is a consequence of a unique infestation with more eggs or of a repeated infestation. We have found out a reduced localization at the level of the following organs: lungs, spleen, choledoch, urinary bladder, kidneys, muscles, each one with a percentage of 0.5.

The distribution of cystic echinococcosis cases hospitalized in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009, shows a percentage of 49.9 cases of individuals with risky occupations (agriculturers, sylviculturists, veterinarians, workers rural) and a percentage of 50.6 cases of individuals who have nothing to do with the rural environment. By the statistical estimation we have found out that there is no statistical significance in the way of presentation of the individuals depending on occupations when they came to hospitalize (tcalculated=0.1 for p>0.01). The results may be explained by:

- The increase of the unemployment rate and the lost of the working places in the urban environment determines the migration of the population to the rural environment, where the factors that support infestation are maintained;
- The existence of a category that, by different means, has caught the disease in the rural environment, but lives in the cities and is recorded there.

Conclusions

1. The epidemiological study plays an important role in the correct diagnosis of human cystic echinococcosis;
2. The number of the investigated cases in the Surgical Clinic no. III of Cluj-Napoca, during 2006-2009, does not certify the reality, because the surgical cases represent only the revealed part of the disease.
3. Cluj-Napoca city is a real centre of treatment and diagnosis of this parasitosis, summarizing cases that come from many counties of Transylvania.
2.2. SEROLOGICAL EVALUATION OF THE SAMPLE OF PATIENTS HOSPITALIZED FOR HYDATID CYST IN THE SURGICAL CLINIC NO. III, DURING 2006-2009

The estimation of a probable diagnosis of cystic echinococcosis is carried out on the basis of some clinical manifestations. In spite of the clinical polymorphism of the disease in its different localizations, the disease follows its course and has at least two constant manifestations: pain and the formation of the benign tumor. If we add the allergic cutaneous syndromes, an important symptomatological triad is completed. The presence of the cyst formation is underlined by imagistic techniques: echography, computed tomography, radiologic examination, magnetic resonance.

The immunodiagnosis, in order to detect serical antibodies, is used to confirm the images that suggest hydatid cyst structures or for the differential diagnosis in case of imprecise tumor images. Indirect immunofluorescence, immunoelectrophoresis, immunoblot test, ELISA immunoenzymatic test are the most used serological tests.

2.2.1. Serological diagnosis of cystic echinococcosis by the ELISA technique, in the cases hospitalized in the Surgical Clinic no. III and Internal Medicine Clinic no. III of Cluj-Napoca, during October 2006–December 2009

Purpose

Human infestation with *E. granulosus* determines an increased production of seric immunoglobulins and the formation of IgG, IgM, IgE and IgA specific antibodies. It is known that IgG is high in the whole current infestations and in any localization, and it persists a different number of years after the ablation of the cyst. The main aim of this study is the estimation of the serological tests in order to establish the diagnosis of cystic echinococcosis.
Materials and methods

- We have carried out serological tests in 104 individuals in order to underline IgG type anti-
  *Echinococcus* specific antibodies:
  
  ✷ 78 individuals hospitalized in the Surgical Clinic no. III of Cluj-Napoca, during October 2006–December 2009; we have carried out a single titration in 60 individuals who were hospitalized for surgical intervention, in 9 individuals the antibody titration was carried out before the intervention and 2 months after the intervention, 7 individuals were hospitalized again 2 years after the surgical intervention, with suspicion of secondary or recidivating hydatidosis and 2 individuals who come to a check two years after the surgical extirpation of the hydatid cyst;
  
  ✷ 26 individuals hospitalized in Internal Medicine Clinic no. III of Cluj-Napoca, during October 2006 – December 2009; 22 individuals were hospitalized for different digestive problems, and 4 individuals come to a check 1 up to 3 years after the intervention for the hydatid cyst.

- In order to establish the immunodiagnosis, we used the ELISA immunoenzymatic test for IgG antibodies, using German NovaTec kits. The results were expressed in NovaTec kit units (NTU). The values below 9 NTU are considered negative, those between 9 and 11 NTU are not convincing and those above 11 NTU are positive.

Results and discussions

Among the 60 individuals who underwent surgical intervention and a single titration, 16 had a positive serology, which means a percentage of 26.7%. In other 26 cases (43.3%) the values of the antibody titer were below cut off (the level of the test that is considered positive), buy yet important, being above 6 NTU. In 3 cases the values of the antibodies were between 8-8.3 NTU, in 9 cases the values were between 9.2-1.06 NTU, and in 14 cases the values were between 7 and 7.8 NTU. The level of the antibodies in these samples is below the positive level given by the kit (11 NTU), but above the negative value, which oscillated around the average of 0.5 NTU. Due to this reason we
think that these values should be considered positive, in the context of obvious clinical and echographic symptoms. Thus, we have a positivity of 70% in the samples discovered with hydatid cyst and serologically tested (42 positive serums of 60 samples).

There are 18 cases (30%) of the 60 investigated serums in which the serological results did not coincide with the state discovered during the intervention. In 9 cases (15%) the serological results were negative, even if the physicians confirmed the presence of the hydatid cyst in the intraoperative state, and in 9 cases (15%) the serological results were positive, even if the physicians did not detect the hydatid cyst formation in the intraoperative state.

In human cystic echinococcosis, the immune response is the results of the permanent diffusion of antigenic substances through the walls of the cyst (Todorov and Stojanov, 1979). These changes are the basis of the structural characteristics of the cystic membrane, which may be intact, fissured, thickened or calcified.

The causes of the false negative results:

- The size and the integrity of the cysts, small hydatides with thickened fibrous capsules;
- The calcification of the cysts appears after the death of the parasite, or determines its death;
- The infection of the hydatid cyst with pathogenic germs due to the biliary-cyst fistulas;
- The immunity status of the patient, in children and old people the level of the antibodies is slightly decreased;
- The existence of local strains, with different capacity of producing antibodies (the bovine strain produces sterile hydatides);
- The localization of the cyst, in pulmonary, cerebral, ocular localization the antibody titer is more reduced.

The causes of the false positive results:

- Individuals with tumors, carcinomas, malignization processes, hepatic cirrhosis;
- Crossed reactions between the echinococcus antigens and the specific antibodies of helminthiasis (filariasis, cysticercosis, hydatid alveococcosis).
We have succeeded to carry out dynamic serological tests in 9 individuals, in the preoperative stage and one or twice in the postoperative stage, 2 months after the surgical intervention. The dynamic serological evolution of the cases is shown in table 5.

In table 5 we may see that there are variations of the level of the antibody titer, which were detected by IgG-ELISA enzymatic test, in the two stages of diagnosis: preoperative and postoperative. In the whole 9 cases the diagnosis of hydatid cyst was certainly established in the intraoperative stage.

Dynamics of anti-*Echinococcus* antibodies in serologically monitored people

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Preoperative test</th>
<th>Postoperative test I</th>
<th>Postoperative test II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg.</td>
<td>NTU</td>
<td>Pos.</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>X</td>
</tr>
</tbody>
</table>

The cases of surgically confirmed cystic echinococcosis, with pre and postoperative negative serological results, in which the level of detected antibodies is constant but much reduced, are included in the situation in which the body does not secrete a significant quantity of specific antibodies in the presence of the hydatid cyst. There are cases in which the pre and postoperative serological results remain negative, but there is a dynamics of the antibodies, in respect of the decrease of their titer, due to the therapeutic success and to the elimination of the antigen source that induced the production of antibodies. We have found similar situations to the decrease of the antibody titer, but both tests are above the positivity borderline of the test. Even if they are negative or positive, the antibody titer values show important signs regarding the evolution in time of those cases. The increase of the antibody titer from values below the positive borderline in the preoperative stage to ambiguous values after the intervention, and then positive again, or from positive values in the preoperative stage, which continue
to increase in the postoperative stage, represent an important argument on behalf of the postoperative dynamic surveillance of the individuals who underwent interventions of cyst eradication. In these individuals it raises the question of a possible secondary cystic echinococcosis by the dissemination of the parasitary elements in the body, possibly during the intervention. There are cases that must take medication treatment and must be followed in time from the serological and imagistic point of view. In the situations in which the antibody titer remains increased much time after the intervention, and the echography does not show the presence of a new hydatid in the body, WHO recommends to apply a secondary serological test, in order to confirm the parasitosis.

Among the 78 individuals hospitalized in the Surgical Clinic no. III of Cluj-Napoca, which we have tested for anti-\textit{Echinococcus} antibodies, 7 come for a new hospitalization almost 2 years after the intervention for the hydatid cyst eradication, due to the reappearance of some clinical signs and symptoms that raise the suspicion of a secondary cystic echinococcosis or of a recidivation.

After the anamnesis, the abdominal ultrasonography, the serological tests and the surgical intervention, we have established the following cases: one case of secondary cystic echinococcosis, four cases of recidivating cystic echinococcosis, one case of secondary cystic echinococcosis combinated with recidivating hydatidosis and one case with a different diagnosis. The situation of the cases that are suspected of secondary or recidivating cystic echinococcosis and that come for a new hospitalization are shown in table 6.
Situation of the cases suspected of secondary and/or recidivating cystic echinococcosis

<table>
<thead>
<tr>
<th>Cases</th>
<th>First hospitalization</th>
<th>Second hospitalization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diagnosis</td>
<td>Ac Titer</td>
</tr>
<tr>
<td>Secondary hydatidosis</td>
<td>CHH segment VII and VIII</td>
<td>-</td>
</tr>
<tr>
<td>Recidivating hydatidosis</td>
<td>CHH segment VIII</td>
<td>IgG=5.6NTU</td>
</tr>
<tr>
<td>Recidivating hydatidosis</td>
<td>CHH segment VI with biliary fistula</td>
<td>IgG=29NTU</td>
</tr>
<tr>
<td>Recidivating hydatidosis</td>
<td>CHH segment VI partially calcified</td>
<td>IgG=2.46NTU</td>
</tr>
<tr>
<td>Recidivating hydatidosis</td>
<td>CHH segment VIII</td>
<td>IgG=1.5NTU</td>
</tr>
<tr>
<td>Operated CHH with case history</td>
<td>CHH segment VII corticalized</td>
<td>IgG=4.7NTU</td>
</tr>
<tr>
<td></td>
<td>CHH segment IV intraparenchimatous</td>
<td>-</td>
</tr>
<tr>
<td>Secondary hydatidosis</td>
<td>CH retroperitoneal latero-iliac</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Among the 78 individuals hospitalized in the Surgical Clinic no. III of Cluj-Napoca, which we have tested for anti-*Echinococcus* antibodies, 2 come for an examination almost 2 years after the intervention for hydatid cyst eradication. Even if the whole operated individuals were advised to come for an examination, few of them have come again due to the fact that their state has been ameliorated. We may note the lack of sanitary education, namely the fact that people are not aware of the importance of postoperative surveillance. In both cases, the antibody titer has decreased, but the value was still positive. Thus, in the first case the titer has decreased from 19 NTU to 18 NTU, and in the second case from 35 NTU to 12 NTU.

26 individuals hospitalized in the Internal Medicine Clinic no. III of Cluj-Napoca, were tested for hydatid cyst in order to carry out a differential diagnosis. Among the 26 individuals, 22 have come to the Internal Diseases Department due to different clinical signs and symptoms, such as: abdominal pains, cutaneous eruptions, migraines, nauseas and vomiting, hepatomegaly, scleral-tegumentary jaundice, etc. Another 4 individuals,
who have come to an examination at the Internal Disease Department 1 up to 3 years after the surgical intervention, have negative serological results, and this shows that the antigen source that induced the production of antibodies was eliminated from the body and the secondary cystic echinococcosis did not appeared. The situation of the serological tests carried out in the individuals hospitalized in Internal Medicine Clinic no. III of Cluj-Napoca, suspected of cystic echinococcosis, is shown in table 7.

Situation of the serological tests in people hospitalized in Internal Clinic III, Cluj-Napoca

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>Hydatidosis</td>
<td>1</td>
<td>16NTU</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6-8.5NTU</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Alveococcosis?</td>
<td>1</td>
<td>11.68NTU</td>
</tr>
<tr>
<td>Hepatic tumors, carcinomas, hepatic cirrhosis, malignization processes</td>
<td>2</td>
<td>6-8.5NTU</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.3-5.5NTU</td>
</tr>
</tbody>
</table>

It results the fact that, for a good diagnosis of the hydatid disease, in order to implement an appropriate therapy, it is not enough a single investigation, but physicians should associate the serological and imagistic data with the anamnesis of the cases. A good cooperation and an exchange of information between the clinical physician and the laboratory should be imposed.

The implementation of postoperative surveillance protocols of the individuals who underwent hydatid cyst interventions, by serological and imagistic tests, whichever the case may be, should be benefic in order to ensure a qualitative medical act and on behalf of the patient.

Conclusions

1. IgG ELISA technique in the case of particular diagnosis, should be associated with other testing systems, in order to confirm the results. The values should be interpreted according to the clinical symptoms;
2. The individuals hospitalized in the Surgical Clinic no. III who underwent a single titration of anti-\emph{Echinococcus} antibodies, have shown the following serological situation: 70\% of the cases were true positive, 15\% of the cases were false negative and 15\% of the cases were false positive;

3. The dynamics of the antihydatid antibodies of the monitored individuals brings important information regarding the evolution of the parasitosis in time, and of the therapeutic success;

4. The serological tests carried out in the individuals who came again for an examination due to new disease symptoms, have shown the existence of 4 cases of recidivating hydatidosis, one case of secondary hydatidosis, one case with different diagnosis and one case of recidivating hydatidosis combined with secondary cystic echinococcosis;

2.2.2 Correlation of the serological results with the results of other investigation methods which were used in the diagnosis of human cystic echinococcosis

**Purpose**

The diagnosis of cystic echinococcosis only by clinical symptoms and echography images is sometimes difficult and confuse. In order to establish correctly the diagnosis it is necessary to corroborate the paraclinical investigations with the serological tests. We have followed the correlation of the serological results with the results of other investigation methods which were used in the diagnosis of human cystic echinococcosis.

**Materials and methods**

- In 78 cases with surgical intervention, the serological results were compared with those of the echography investigations and of unspecific investigations, such as the level of eosinophiles and leukocytes;
- The echography images and the values of the eosinophiles and leukocytes were taken from the observation charts of the patients;
In order to establish the immunodiagnositics we have used the ELISA immunoenzymatic technique for IgG antibodies, working with German NovaTec kits. The results were expressed in NovaTec kit units (NTU). The values below 9 NTU are considered negative, those between 9 and 11 NTU are not convincing and those above 11 NTU are positive.

**Results and discussions**

The investigated individuals had symptoms that raised the suspicion of cystic echinococcosis, such as:

- Intermittent pain with different intensity in the right hypochondrium, epigastrium, periumbilical;
- Inappetence, morning vomiting, post-prandial ballooning, bitter taste, pyrosis, acholic stools;
- Shivers, intermittent fever;
- Scleral-tegumentary jaundice, pruritus, anaphylactic episode;
- Fatigability, asthenia, decrease of the working capacity.

There were also asymptomatic individuals, and the cyst formations were discovered during the routine echography examination.

Then the clinicians and the laboratory cooperated in order to establish the diagnosis.

The echography investigations have underlined:

- Cyst formations with or without thickened walls;
- Septal or vesicular structures;
- Hypoechogenic or hyperechogenic areas;
- Well delimited or diffuse delimited homogenous transonic images.

After the clinical stage we could not establish for sure each time if the cyst formation discovered by echography had a hydatid source.

We have carried out approximate paraclinical investigations: eosinophilia and leukocytosis, but they did not give a sure diagnosis, but only one of high probability. Simultaneously with these investigations we have carried out serological tests for IgG/anti-*Echinococcus* specific antibodies. The results of the serological tests have
underlined a number of 26 (33.3%) positive cases with the values of the kit units (NTU) above 11. In other 30 cases the values of the antibody titer were below cut off, but yet important, being above 6 NTU. In 14 cases the level was above 8 NTU, being very close to the unsure level. The level of the antibodies in these samples is below the value considered as positive by the kit, but above the value of the negative reference sample, that oscillated around 0.5 NTU. If we take into consideration these values, corroborated with the echography images, the level of seropositivity becomes 71.8% (56 positive from the 78 cases).

The correlation of the serological results with the results of other investigation techniques has shown the fact that we have underlined the cyst formation by echography in the whole individuals with the diagnosis oriented towards hydatidosis, but:

- In 19 cases of the 78 serologically tested cases, the diagnosis of hydatid cyst was infirmed in the intraoperative stage (5 cases of serous hepatic cysts, 1 case of biliary serous cyst, 1 case of spleen serous cyst, 1 case of ovarian serous cyst, 1 case of pancreatic tumor, 1 case of abdominal hernia, 1 case of hepatic abscess, 1 case of hepatic cirrhosis, 1 case of toxic shock syndrome, 1 case of hepatic cell carcinoma, 3 cases of hepatic neoplasm, 1 case of metastasis colon neoplasm).

In 9 cases the serology was positive, 7 cases (9%) being accompanied by hyperleukocytosis, and 2 cases (2.6%) with the eosinophiles and leukocytes within normal values. The rest of the 10 cases had negative serology with hyperleukocytosis (7 cases, 9%) or with leukocytes and eosinophiles within the normal values (3 cases, 3.8%).

- In 59 cases the existence of the hydatid in the body was confirmed in the intraoperative stage. In these cases we have the following situation:
  - In 4 cases (5.1%) the positive serology was associated with hypereosinophilia (eosi
    nophiles >5%);
  - In 19 cases (24.3%) the positive serology was associated with hyperleukocytosis (leukocytes > 8K/uL);
  - In 8 cases (10.2%) the three parameters were associated;
  - In 16 cases (20.5%) the positive serology was associated with leukocytes and eosinophiles within normal values;
- 3 cases had high level of eosinophiles and no positive serology and hyperleukocytosis, 3.8%;
- In 4 cases none of the parameters exceeded the normal values, 5.1%;
- 4 cases had hyperleukocytosis with negative serology and normal eosinophilia, 5.1%;
- One case had hypereosinophilia and hyperleukocytosis and no positive serology, 1.3%.

Among the investigated cases (78 tested serums), 7 individuals (9%) had hypereosinophilia, 37 individuals (47.4%) had hyperleukocytosis, in 9 individuals (11.5%) the two parameters were increased and associated, and in 25 individuals (32%) the values of the two parameters were within the normal values.

Most of the eosinophiles were discrete (5-10%) and were found in the cases in which the cysts reached the final stage of development. The maximal values of eosinophiles were between 14-36.5%, and consequently high enough and may be explained by the existence of a secondary cystic echinococcosis. Therefore, the determination of eosinophilia could not give enough arguments and information regarding the hydatid disease, but anytime it appeared a constantly high eosinophilia, which did not have another explanation, it could point out the existence of a cyst in the human body.

We have found a high level of leukocytes both in positive serology individuals and in negative ones, so that 47.4% of the investigated cases had hyperleukocytosis. In most of the cases of hyperleukocytosis, the values of the leukocytes were moderate, namely between 8-10 K/uL. In the cases in which the values of the leukocytes were above 10 K/uL (11-20 K/uL), it was either a complicated recidivating hydatid cyst or another pathology. In the cases in which after the intervention we have not detected hydatid cyst formations, hyperleukocytosis together with some clinical and epidemiological data represented the element which helped us to determine the diagnosis.

The lack of the whole signs that could guide us towards a hydatid disease appeared in 7 individuals (9%) of the investigated cases. In 4 individuals (5.1%) the presence of the hydatid cyst was confirmed during the intervention. There were thickened walls and
well determined cysts, partially or totally calcified cysts, in which the exchange with the exterior was reduced.

Conclusions

1. The correct diagnosis of cyst echinococcosis in the human host involves the corroboration of the epidemiological data with clinical signs, with imagistic aspects and with the serological results;
2. The sanguine eosinophilia and leukocytosis are guiding elements which help to determine a correct diagnosis, and their absence does not exclude the possibility of the existence of the parasite;
3. The serological methods, bases on the knowledge of the immunity mechanisms of the hydatid disease, give the indirect diagnosis of the hydatid cyst.

2.3. MEDICAL AND SOCIAL IMPORTANCE OF HUMAN CYSTIC ECHINOCOCCOSIS

Human cystic echinococcosis is a fearsome disease which affects the vital organs, with clinical latency and tardive diagnosis, in advanced morphological stages, with therapeutic limits and prognosis that are not always favourable. But there are some important signs that must be followed in people diagnosed with hydatid cyst:

The complications of the disease, 11.4% of the cases hospitalized in the Surgical Clinic no. III of Cluj-Napoca during 2006-2009 had the following complications:

- Biliary fistula;
- Tear of the hydatid cyst in the biliary duct and the peritoneal cavity;
- Sclerosing cholangitis;
- Toxic shock;
- Lithiasic cholecystitis;
- Suprainfected hydatid cyst;
- Hepatic abscess;
- Eventration;
- Adherential syndrome.

**Hydatid cyst in children**, the evolution of the disease in children is usually favourable. But, we have to attach importance to the hydatid disease in children because it entails consequences in the family environment, by emotional distortion and by the economic and social issues of those involved.

**Duration of hospitalization**, the 185 hospitalized cases needed 195 hospitalizations, totalizing 1964 days of hospitalization. The average of the days of hospitalization was 10.1 days/case. 5.4% of the cases of hospitalized hydatid cyst needed several hospitalizations, their number varying between one and two per case.

**The size of the cysts**, the cysts varied from several centimeters to more than 15 cm. We have noted that the size of the hydatid cysts was bigger in children and young adults.

**The basic treatment** is the surgery, combined with chemotherapy in pre and postoperative stage. Blocking the secondary cystic echinococcosis in the incipient stage with medication treatment, without reaching repeated hospitalizations and new surgical interventions, with social, financial and emotional consequences of those involved, would be both for the benefit of the individuals and of the society, due to the reduction of the temporary working incapacity and of the increased costs of prolonged and multiple hospitalizations.

According to Duca et al. (1993), **the mortality** due to hydatid cyst in Cluj county is of 8.1%, and the average mortality rate calculated by Coroiu (1998) is 0.29/o000. Studies regarding the hydatid cyst carried out on corpses would bring real information regarding the prevalence of the hydatid cyst.

The implementation of preventing measures must be adopted in order to reduce the risk represented by this parasite for the health of the population.
3. FACTORS THAT SUPPORT *E. GRANULOSUS* INFESTATION IN THE INVESTIGATED AREA

There are many factors that condition *E. granulosus* infestation and they are related both to the environment and to the parasite and its host.

Here we list some of the most important factors that determine the frequency of the parasite in the area we have investigated:

- Ecological conditions;
- The environment polluted with excrements resulted from infested dogs;
- Social-economic conditions;
- Cultural and behavior factors;
- Human negligence, lack of sanitary education, eating habits;
- The dimension and fluctuation of the host: transhumance, permanent circulation of a part of the population from the urban environment to the rural one;
- The professional risk.

4. CONSIDERATIONS REGARDING THE STATUS OF THE HUMAN CYSTIC ECHINOCOCCOSIS IN CLUJ COUNTY, WHICH IS A PART OF THE NORTH-WESTERN AND CENTRAL AREA OF ROMANIA

Synthesizing the results and based on bibliographic data, we may underline briefly the status of the human cystic echinococcosis in the north-western and central part of the country.

- The high level of antihydatid antibodies, 5.12%, in apparently healthy population, is a proof of the infesting potential of the environment and of the existence of an acquired immunity, which is characteristic to the endemic areas;
- A level not at all insignificant of the prognosticated disease (2.59%) based on the probabilistic value of the positive test;
- The presence of the parasite in the whole area is a proof of the existence of the perpetuating conditions of its biologic cycle with direct consequences over its transmission to the humans;
• The high level of seropositivity in the whole categories of population is the proof of the endemicity;
• A higher parasitation level in some counties proves the zonal distribution of the parasite in the studied area;
• The frequency of cystic echinococcosis in children shows the most recent rate of transmission of the parasite, a proof of its maintenance in the area.

The endemicity of the parasitosis in Cluj county is proved by:
• The value of the multiannual average morbidity (2000-2009) of 4,6o/oooo;
• The linear evolution tendency, slightly ascendant for a 28 years period (1981-2009);
• A level of anti-\textit{Echinococcus} antibodies in asymptomatic population of 4,35% with a hydatid disease prognosis of 2,02;
• The presence of the hydatid cyst in children (5-19 years old) with parallel evolution of the disease in children and adults;
• The existence of multiple localizations of the hydatid in the surgical cases;
• Annual incidence of more than 10 cases per 100,000 inhabitants in the rural environment proves the hyperendemic character of the disease in certain years;
• The higher frequency of the disease in individuals with risky occupations, and the presence of a higher percentage in individuals who apparently have nothing to do with it.

5. INTERPRETATION OF THE IMMUNODIAGNOSTIC IN THE PARTICULAR TESTED CASES AND ITS PLACE IN MONITORING THE EVOLUTION OF THE HYDATID DISEASE

Synthesizing the results obtained after we have applied ELISA test in order to detect anti-\textit{Echinococcus} antibodies in hospitalized samples and based on bibliographical data, we try to underline the place of the immunodiagnostic in the pathology of human cystic echinococcosis.
In case of particular serodiagnostic it is necessary to optimize the results, namely the negative serological results should be true negative, so that they could not fail the diagnostics, and be true positive;

The interpretation of the ELISA serological test must be corroborated with the clinical symptoms, with the information given by the imagistic techniques, with the hematological results and with the anamnesis of the patients;

During the monitoring of the hydatid disease it should exist a leveling of the serological diagnostic. The suspected serum must be detected with very sensible methods (primary tests), and then they must be confirmed with other methods, which have a very high specificity (secondary tests) (table 8).

Steps of the serological investigations in the cases suspected of cystic echinococcosis

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| First step – primary antibodies test |
| Testing in order to detect serum antibodies: IgG-ELISA with *E. granulosus* antigens or other appropriate techniques (latex agglutination, indirect hemagglutination, immunoelectrophoresis) |

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<th>Next steps</th>
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<tr>
<td>Seronegative samples</td>
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<td>Individuals without imagistic structures that could suggest hydatid cyst</td>
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<td>-other serological tests are not needed</td>
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5. CONSIDERATIONS REGARDING THE FIGHT AGAINST CYSTIC ECHINOCOCCOSUS

Any parasite preventing, combating and elimination action should include two important stages:

- The stage of epidemiological surveillance of the area, which includes:
  - Attentively control regarding the distribution of the infestation, by inventorying the operated cases;
  - Determination of the antihydatid antibodies in healthy population;
  - Determination of the frequency of the parasitosis in the final hosts and the intermediary hosts;
  - Evaluation of the social, the cultural and the environmental factors that determine the presence of the parasite or its dissemination.

The results of our study correspond to this stage.

- The stage of prevention and disease combating, which implies:
  - Reduction of canine infestation;
  - Limitation of the number of homeless dogs;
  - Avoiding infestation or reinfestation of the final hosts;
  - Quick inactivation of the infested organs;
  - Rigorous personal hygiene;
  - Early and sure diagnosis;
  - Postoperative surveillance of the patients;
  - Sanitary education.
CONCLUSIONS

1. The parasite we have studied on the basis of the biological, the epidemiological and the serological parameters is characterized by: active and recent transmission; similar and homogenous infestation risk; continuity in its transmission; high level of parasitation in humans (surgical morbidity, frequency of the hydatid antibodies, disease prognosis).

All these prove *E. granulosus* endemic state in the central and north-western part of Romania.

2. The categories of population with higher infestation risk, which were underlined on the basis of our study, are: rural population, females, adults, children.

3. Cluj-Napoca city is an important center for the treatment of the human hydatid disease in the central and north-western part of the country.

4. Taking into consideration the double epidemiological potential of the disease, by its coexistence in natural and synanthropic focuses, the surveillance of the evolution of the parasitosis in humans must not be abandoned even if the momentary prognosis seems to be favourable.

BIBLIOGRAFIE SELECTIVĂ
SELECTIVE REFERENCES


