Author's response to reviews

Title: Incidence of human cystic echinococcosis in Slovenia

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The manuscript was revised according to the reviewers' comments. The authors' responses to the reviewers' comments are as follows.

Response to Dr. Thomas TR Romig

Major Revisions

1) Do all 34 patients show imaging sign compatible with CE?
Yes, all 34 patients show imaging signs compatible with CE; so, in the abstract and in the results section, following your suggestion, »other symptoms (jaundice, pain..)« which are not diagnostic are omitted.

2) Why were the sera with pattern P4 and P5 not studied further?
This study partially overlaps with our previous study. We didn't mention our previous research of alveolar echinococcosis in submitting the present paper because our plan was to focus our research only to cystic echinococcosis. We agree with you that it should be mentioned; therefore, the Results section on page 4, third sentence was corrected and the reference of our previous article was added as follows: »Eight sera with patterns P4 were already confirmed in our previous study to be positive for alveolar echinococcosis. Six sera with pattern P5 with no possibility to discriminate between two species were not studied further (Ref. 3 - Logar J et al., 2007)."

3) With reference to your suggestion, we used, in the Conclusions and in Discussion sections, the term »prevalence for the five-year period« instead of the term »incidence of CE«.

4) In the Discussion section, in the last paragraph, we explained that the detected cases by serological methods cannot be allocated to the definitive lifecycle of CE:
»Most forms of human CE are transmitted in domestic lifecycles involving dogs and livestock. The most known is the sheep-dog cycle. In the last years, it has..."
been recognised by genetic characterisation that, in the genus of Echinococcus there are distinct species and strains (e.g. sheep, pig, cattle, cervid, camel strain) based on morphology, host specificity and molecular characteristics [10]. In the 1970's, Brglez estimated that the pig (its breeding is also nowadays the most wide-spread in the eastern parts of the country) was the most important intermediate host in epidemiological and epizootiological conditions of CE in Slovenia [11]. Because it is impossible to allocate the detected CE positive cases to any of the above mentioned forms by serological methods used in our study, we can only conclude that our patients' have been infected by one of species/strains of Echinococcus granulosus complex «.

1) Term E. granulosus complex is mentioned in the sentence added to the Discussion section.

2) The prevalence of 4.8 was based on the total population of Slovenia. So, the corrected sentence is: »In 1956 to 1968, the prevalence of CE in the total population of Slovenia, was 4.8 per 105 inhabitants.

3) Mistake in the abstract: Eggs nested in the liver.. is corrected in new sentence: In the intestine the larvae released from the eggs are nested in the liver, lungs or other organs of livestock as intermediate hosts and humans as abberant hosts.

4) Background: The Conclusions section is modified: In the study, it was found out that human CE was mostly spread in the same areas of Slovenia as in the past, but its prevalence decreased from 4.8 per 105 in 1956-1968 to 1.7 per 105 inhabitants in the period 2002-2006. In spite of the decreased prevalence of CE in the last years, it is suggested that the clinicians and public health authorities, especially in the eastern parts of Slovenia where the most CE patients come from, pay greater attention to this disease in the future.

There are no known data of CE in neighbouring countries.

Response to Dr. Fabrizio Bruschi

Major Revisions

Taking into account your suggestion, we added lines (/) marking all serologically examined patients for CE on the map of the origin.
The risk factor is not mentioned, because it is not known; therefore in the Discussion section stresses only the importance of controlling local slaughtering of livestock, general and sanitary attitude of livestock breeders, providing safe drinking water, and controlling and providing anthelmintic treatment of dogs population.

Yes, criteria to consider low positivity are indicated by the manufacturer.

No data obtained by other methods (ELISA IFT..) are provided because, in our lab, we use only IHA test for screening of echinococcosis; so, we have no data of the other tests.

Out of IHA positive sera that were not confirmed by WB, 74 sera were positive in the low titre 1:32-1:128, 4 were positive in the titre 1:256 and 1 in the titre 1:512. We cannot explain such level of false positivity; maybe it is due to cross-reactivity with other parasites, but these data are not mentioned in the protocol of Dade Behring test.

Explanation why in the Results section, it is stated that eight sera with patterns P4, and 6 sera with pattern P5 were not studied further.

This study partially overlaps with our previous study. We didn't mention our previous research of alveolar echinococcosis in submitting the present paper because our plan was to focus our research only to cystic echinococcosis; but, we agree with you that it should be necessary to mention it. Therefore, the Results section on page 4, third sentence was corrected and the reference added as follows: » Eight sera with patterns P4 were already confirmed in our previous study to be positive for alveolar echinococcosis. Six sera with pattern P5 with no possibility to discriminate between two species were not studied further (Ref. 3 - Logar J et al., 2007).

Results of confirmed cases might be summarised in a table.

We believe that the table with the percentage of different IHA titres and Patterns of WB is not necessary because, in the study, we didn't compare the quality of two tests, but we tried to find out what was the prevalence of human CE in our country.

Discussion section was modified.

Minor revisions

Page 3, Line 8: 5 references were reduced to 1 reference.
Line 8: the term »cystic« was added.
Line 20: the term »higher« was added.
Page 4, Line 1: the year (2000) was added.
Page 5, Line 17: »or re-emergence« was deleted.
Response to Dr Franz Josef Conraths

Major Revisions

i) The sensitivities of different tests in the patients with a liver manifestation of echinococcosis as compared to the patients with a manifestation in the lungs were taken into consideration in our analysis. One sera of patients with lung echinococcosis was IHA positive in the titre 1:256 and other in the titre 1:512; both were also confirmed by WB.

ii) The sensitivity and specificity of the IHA is not mentioned in the protocol of Cellognost-Echinococcosis, Dade Behring test. We therefore did not mention it in our report. Cut-off titre of IHA test is 1:16. According to test protocol, low titres 1:32-1:128 are of the 'suspected' range.

Following your suggestion, the clarification of the status of 79 sera was added to the Results section, second sentence:

> Out of IHA positive sera that were not confirmed by WB, 74 sera were positive in low titres 1:32-1:128, 4 sera were positive in the titre 1:256 and one in the titre 1:512.

iii) Fig. 1. Lines (/) mark the places from where the patients' sera were obtained and the places of CE negative cases, while asterisks (*) mark the area where the patients tested for CE were serologically confirmed.

iv) Data on treatment is missing.

We agree with you that histological data are required to reach a definitive diagnosis of CE. Because only a few Echinococcus cysts were sent to examination to our Institute, we didn't include these cases in the study. We believe that the serological data are sufficient for the information on the prevalence of cystic echinococcosis in Slovenia.
Response to Dr Abdollah Rafiei

General / Major Revisions

As very little has been published on cystic echinococcosis in this part of Europe (the area of former Yugoslavia), we think that this paper deserves to be published in international medical journal and not in a local one.

We agree with you that, in epidemiology, incidence means the rate of occurrence of new cases. Therefore, we changed the title of our study to »Human cystic echinococcosis in Slovenia«

In page 6, we corrected the sentences not related to the results of current study.