

The authors thank the editors and reviewers for their valuable comments. Based on their suggestions we performed revisions and in the following responses critically discuss all the points raised. The appropriate changes are now included in the text and are given below. We believe that these have considerably strengthened the manuscript. The changes are addressed in a point-by-point fashion.

Reviewer 1 (No. 2720326)

Introduction

1. You state the liver to be the first organ affected by metacestode development. Actually, this is mostly, but not always the site of primary infestation.

We agree with the reviewer that the liver is not always the first organ affected by metacestode. In our patients, however, only 1% are having no primary liver manifestation.

2. Please use the correct terms, i.e. 'Echinococcus alveolaris' (not written in italics) or 'metacestode stage of E. multilocularis' instead of just 'E. multilocularis' when you write about the metacestode.

As suggested by the reviewer appropriate terms have been corrected.

3. You use the term 'HAE' without explaining the abbreviation. Since you only use it once, an abbreviation is not necessary.

As suggested by the reviewer the term HAE has been spelled out.

Results

4. n=25 is 13.5%, not 23.5%. You should use 13.0% instead of 13%.

The typing error has been corrected

Discussion

5. In the 4th paragraph, you use $p < 0.005$, while you wrote 0.05 in the results part.

The error in the discussion has been corrected

Table 1

6. 85/185 is 45.9%, not 46.0%

The typing error in the table has been corrected

Reviewer 2 (No. 3338507)

1. The research is based primarily on the experience of one radiologist, who analyzed the images. The expertise of this person is emphasized twice in the “methods”. This presents two important limitations in the study, which have not been reported in the manuscript:
 - a. i. Checking or improving the reliability of this study has never been an issue for the authors. I believe that at least two radiologists, or other experts, should independently evaluate the images. It is important that the inter-rater reliability is measured and documented in such a manuscript. This could change dramatically the results of the study, or lead to a more successful classification of the unclassified cases.

The authors agree with the reviewer that the inter-rater reliability presents a problem. The classification was created as part of discussion processes and not according to strict criteria of a double-blind evaluation. A paragraph on limitations of the study has been added in to the Discussion and this issue has been discussed there.

- b. ii. Diagnosing an AE is important for radiologists and physicians who provide their services in rural areas or often small hospitals. An AE specialist is rarely the first person to diagnose such a condition. This means that an ultrasonographic classification should be a tool that should address these needs and should take into consideration the capacities of these physicians. In case only hepatic US experts are able to use this tool, the validity of the tool and the study could be questioned. Most probably such issues have been discussed among the study team. Such limitations should be explicitly referred in the manuscript.

We agree with the reviewer, that it is difficult to diagnose AE. However, knowledge of these classifications should facilitate the diagnosis in many cases.

- c. iii. There is a problem regarding the generalizability of the study. The authors parallelize the results of their work to the WHO CE US stages. I agree that there are a lot of similarities. A major difference though, is that the CE stages present not only morphologically different lesions, but primarily different stages of activity of the CE lesions. This has major consequences regarding the treatment and the prognosis of the person suffering from CE. In the case of this study there is no reference to such evidence.

We agree with the reviewer that there is a difference between the WHO's CE classification and our AE classification because our classification does not directly lead to therapeutic consequences.

- d. iv. No reference to correlations to the morphological findings of different imaging techniques, for instance MRI (Kodama).

The work of Kodama et al. has been discussed in detail in the introduction.

"In 2003, Kodama et al. introduced a five-part classification for assessing hepatic AE with magnetic resonance imaging (MRI)[25]: Type 1: Multiple small round cysts without a solid component; Type 2: Multiple small round cysts with a solid component; Type 3: A solid component surrounding a large and/or irregular pseudo-cyst with multiple small round cysts; Type 4: A solid component without cysts; and Type 5: A large cyst without a solid component. No corresponding classification has yet been published for either CT or US."

2. Little polishing in English needed, but the potential is good.

As recommended by the reviewer the entire manuscript has been checked by a native speaker.

3. Table of abbreviations needed.

As recommended by the reviewer a list of abbreviations has been added into the manuscript.

4. Title: The main and short titles accurately reflect the major topic and content. The abbreviation AE could be introduced in the title.

As suggested by the reviewer the abbreviation AE has been included into the title.

5. Abstract: The abbreviation AE shall be introduced either in the title or in the abstract.

The abbreviation has been introduced both in the title and in the abstract.

6. How has the classification scheme been developed? The rarity of the condition has been underlined at least three times in the manuscript and once in the abstract, without providing any numbers. I would like to be informed about the prevalence and incidence of the condition for instance in endemic regions.

The University Hospital Ulm has for many years been the center for this disease in Germany. Several studies have shown that prevalence rates have been increasing in certain regions. For example, recent parasite density estimates in southwestern Germany were 10× higher than estimates before 1990 (1, 2, 3), and unexpectedly high prevalence rates in several urban fox populations have been reported (4). The combination of increased fox populations and increased parasite prevalence within these populations has led to a considerable increase in the overall parasite biomass per surface unit. Hegglin et al reported 102 human cases between 1981 and 2000 in Germany (5, 6). Most cases are clustered in the southern states of Baden-Wuerttemberg and Bavaria (5). Today this parasite has been confirmed in all regions of Germany (7). However, high prevalence exceeding 30% are mainly reported from fox

populations in the south of the country (7). In northern Germany areas of high endemicity appear to be interspersed focally in low endemicity regions [8, 9]

- [1] Jenkins DJ, Romig T, Thompson RCA. Emergence/re-emergence of *Echinococcus* spp. — a global update. *Int J Parasitol.* 2005;35: 1205–19.
- [2] Romig T. Spread of *Echinococcus multilocularis* in Europe? In: Craig P, Pawlowski Z, editors. *Cestode zoonosis: echinococcosis and cysticercosis*. Amsterdam: IOS Press; 2002. p. 65–80.
- [3] Schweiger A et al. Human alveolar echinococcosis after fox population increase, Switzerland. *Emerg Infect Dis.* 2007 Jun;13(6):878-82.
- [4] Deplazes P, Hegglin D, Gloor S, Romig T. Wilderness in the city: the urbanization of *Echinococcus multilocularis*. *Trends Parasitol.* 2004;20:77–84.
- [5] Kern P, Bardonnnet K, Renner E, Auer H, Pawlowski Z, Ammann R, Vuitton DA, Kern P, Registry EE: European echinococcosis registry: human alveolar echinococcosis, Europe, 1982–2000. *Emerg Infect Dis* 2003, 9:343-349
- [6] Hegglin D, Bontadina F, Gloor S, Romig T, Deplazes P, Kern P. Survey of public knowledge about *Echinococcus multilocularis* in four European countries: need for proactive information. *BMC Public Health.* 2008 Jul 21;8:247.
- [7] Romig T, Bilger B, Mackenstedt U: [Current spread and epidemiology of *Echinococcus multilocularis*]. *Dtsch Tierarztl Wochenschr* 1999, 106(8):352-357
- [8] Berke O, Keyserlingk M: [Increase of the prevalence of *Echinococcus multilocularis* infection in red foxes in Lower Saxony]. *DTW –Deutsche Tierärztliche Wochenschrift* 2001, 108: 201-205.
- [9] Tackmann K, Löschner U, Mix H, Staubach C, Thulke H-H, Conraths FJ: Spatial distribution patterns of *Echinococcus multilocularis* (Leuckart 1863) (Cestode: Cyclophyllidae: Taeniidae) among red foxes in an endemic focus in Brandenburg, Germany. *Epidemiol Infect* 1998, 120:101-109

7. There is no reference to limitations, reliability / validity in the abstract.

We don't understand the question

8. Materials and Methods: The method used is in general sufficient for this type of study. However it is not clear enough why this type of categorization was chosen in advance. It looks like having the categories – results prepared in advance, and attempting to build a study around this model. I believe that the team has worked very well, ending up to these five categories, but the manuscript does not describe how and why they chose them in the methods section.

The classification was developed on the basis of many years of working experience and scientifically examinations regarding alveolar echinococcosis.

Reviewer 3 (No. 3314022)

The attached file can not be opened correctly, so that the comments are highlighted but the comments themselves are not visible

1. the structure of the manuscript is not clear in some points

As recommended by the reviewer the structure of the manuscript has been clarified.

2. the specific aims of the classification are not well stated at the beginning of the manuscript and the reader needs to wait until the discussion for this

The aim of the study has been clear formulated in the abstract and in the introduction:

Abstract: Objective was to establish an ultrasonographic classification based on a large sample of patients with confirmed hepatic alveolar echinococcosis.

Introduction: Objective of the present study was to establish an ultrasonographic classification based on a large sample of patients with confirmed hepatic AE as a way of facilitating the diagnosis, interpretation, classification and comparison of ultrasonographic findings of the rare disease entity.

3. from a methodological point of view, it is unclear whether the Authors classify lesions of patients (and why one or another)

As described above, the classification was developed on the basis of many years of working experience and scientifically examinations regarding alveolar echinococcosis.

4. very importantly, the entire work would be much more complete and of scientific impact if the proposed classification would be tested by evaluating the inter- and intra-observer agreement after different operators have blindly classified US images based on this classification. Even better with the inclusion of operators outside the group following these cases and therefore knowing the cases well, thus reducing the possibility of real blinding. I consider this a major point for revision and I would strongly encourage this type of analysis before the publication of the work. If not possible, the Authors should address this point in the discussion

The authors agree with the reviewer that the inter-rater reliability presents a problem. The classification was created as part of discussion processes and not according to strict criteria of a double-blind evaluation. A paragraph on limitations of the study has been added in to the discussion and the issue has been discussed there.

Comments in the word file:

5. Introduction p. 1 line 4: "Northern Hemisphere" Capital letters needed?
The term has to be written with initial capital letters
6. Introduction p. 2 line 4: The abbreviation HAE not spelled-out before
As suggested by the reviewer the abbreviation has been spelled out
7. Introduction p. 2 line 4-5: following sentence should be rephrased for clarity "Lesions characterized by vesicles and small cysts show a high degree of correlation between 18F-FDG-PET and CEUS findings[24]".
As recommended by the reviewer the sentence has been formulated more understandable
8. Are there any pathognomonic US signs that would facilitate the diagnosis (like in CE) or this classification would just aid the clinician/radiologist to put AE among their differentials?
The hailstorm and pseudocystic pattern are pathognomonic. The ossification, hemangioma-like and metastasis-like pattern help to better classify the disease. The same applies also in the WHO CE classification. The stages IV and V are certainly not pathognomonic and are only possible differential diagnosis.
9. Please clarify very well the aims of the classification (also here and not just in at the end of the discussion) and how this will fit into the diagnostic algorithm of AE
As recommended by the reviewer the aim of the classification has been completed in the introduction:

"Objective of the present study was to establish an ultrasonographic classification based on a large sample of patients with confirmed hepatic AE as a way of facilitating 6: the diagnosis, interpretation, classification and comparison of ultrasonographic findings of the rare disease entity"
10. In the discussion you indicate among further studies to explore any indication/prospect that this classification may reflect also different biological behaviour of the parasite. Maybe worth mentioning it here among the possible aims?
Depending on the new classification, our research group has not performed any investigations regarding the impact of the classification on possible different biological patterns of the disease. This is difficult on the basis of retrospective data.
11. Methods p. 7 line 1: „mean“ would scientifically read better than “average”
As suggested by the reviewer average has been changed into mean
12. Methods p. 7 line ("The remaining 200 patients were excluded from this re-evaluation due to limitations in image quality impacting interpretation or incomplete data sets."): I understand 200 comes from 385 – 185, but please rephrase for clarity (inclusion/exclusion criteria)
As recommended by the reviewer the sentence has been formulated more clearly:

“A total of 200 patients were excluded from this analysis due to limitations in image quality impacting interpretation or incomplete data sets.”

13. Methods p. 7 line 10 (“..... were re-interpreted by a single reviewer....”)Who? Please indicate initials in parenthesis. Also, I would strongly suggest to have this classification blindly tested by different personnel and by the same people multiple times, to assess inter and intra-variability.

As suggested by the reviewer the initials of the examiner has been added in to the appropriate section. Regarding the issue inter and intra rater variability please see point 4 above.

14. Please rephrase for clarity (“The diagnosis of AE was made in cases with unequivocal seropositivity, positive histological findings following diagnostic puncture or partial resection of the liver, as well as findings typical for AE in either US, CT, MRI or PET-CT[15].”): what combination of criteria are needed to classify AE cases as confirmed, probable or possible.

We assume that the classification made by Brunetti (1) has been described in detail in the text. We have clarified it. Our case definition was based on the definition of Brunetti.

[1] Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar Echinococcosis in humans. Acta Trop 2010; 114: 1-16

15. Methods p. 7 (“According to modified WHO criteria of Brunetti et al. 79 cases were confirmed by positive histopathology.....”) this needs a reference.

The appropriate reference has been added

16. Methods p 7. (“According to modified WHO criteria of Brunetti et al. 79 cases were confirmed by positive histopathology and proven specific ELISA from tissue samples.”): Can you explain a bit better what technique is this?

Work-up of the resection or biopsy specimen was done according to standardized histological techniques on paraffin section of formalin fixed tissue. In brief, the tissue was fixed overnight in 5% buffered formalin (pH 6) and then embedded in paraffin. The paraffin slides of a thickness of 3 micrometer were stained with haematoxylin and eosin (H&E) and Periodic acid-Schiff (PAS) according to standard protocols. In addition, immunohistochemistry was done on paraffin sections using the monoclonal mouse antibody Em2G11 (IgG1); for antigen retrieval, the sections were heated in citrate buffer at pH 6 in a microwave oven for 20 minutes. The primary antibody was used in a concentration of 0.2057 mg/ml in phosphate buffer saline; as detection system the EnVision Kit (Dako, Carpinteria, CA, USA) was used according to the manufacturer's protocols (for further details see Barth TF et al PLoS Negl Trop Dis. 2012;6(10):e1877. doi: 10.1371/journal.pntd.0001877. Epub 2012 Oct 25.)

The importance of the ELISA method was published by our research group in Turk Journal of Gastroenterology (Wuestenberg J, Gruener B, Oeztuerk S, Mason RA, Haenle MM, Graeter T, Akinli AS, Kern P, Kratzer W. Diagnostics in cystic echinococcosis: serology versus ultrasonography. Turk J Gastroenterol. 2014 Aug;25(4):398-404).

17. Methods p. 7 (*"Further 85 patients were probable cases with positive serology in two different procedures and positive imaging for AE in two imaging techniques and 21 patients were possible cases with a positive medical history and a positive result for imaging and serology"*). Do you mean by 2 different tests or in 2 different occasion?
We agree with the reviewer that our statement lead to misunderstandings. As recommended we have clarified the statement.
18. Methods p. 8 („The AE-lesions were divided into six morphological patterns. “). 5 or 6? From the graph I guess the 6th pattern is „unclassifiable“, but this is not really a pattern... please explain better.
We agree with the reviewer that there are five patterns. The typing error has been corrected
19. Methods p. 9, statistical analysis (*"One-way ANOVA followed by a Post hoc test (Tukey HSD) was applied to analyze between and within the groups."*). To analyze what? In the results see only a „between“ groups assessment
As correctly noticed by the reviewer only the comparison between the groups has been performed. This has been corrected accordingly in the appropriate sentence.
20. It is evident from these data that you classified 1 lesion per patient. However table 1 shows that a proportion of patients have multiple lesions. Please clarify how you deal with multiple lesions with different patterns, if you observe them, or discuss if you don't. You briefly covered this issue in the paragraph below, but it is quite unclear why you prefer to classify patients rather than single lesions as it is done for CE...is there any reason related to clinical decision making that would derive from the classification of the lesions/patient in toto?
In 80% of our study population 1-3 focal hepatic AE lesions occur. Patients with different AE sonomorphological patterns represent a rarity in our patients.
21. Results (*"In terms of their mean diameter, the hailstorm lesions measured 59.6 ± 27.9 mm; the pseudocystic lesions, 120.0 ± 47.3 mm; the hemangioma-like lesions, 68.1 ± 37.3 mm; the ossification lesions, 28.0 ± 19.4 mm; and metastasis-like lesions, 35.3 ± 33.1 mm (figure 2)"*). Do you mean figure 6?
As correctly noticed by the reviewer figure 6 is meant. The typing error has been corrected.
22. Discussion: If not required by the Journal format, there is no need to spell out and make acronyms again here if done before in the text
As suggested by the reviewer the abbreviations have not been spelled out again in the discussion section.
23. *"The so-called "hailstorm" and "pseudocystic" patterns were described as early as 1984 by Didier et al. "* → Reference needed here as well
We agree with the reviewer that in this sentence the reference is missing. The correct reference has been inserted.

24. *“In conclusion, 95% of cases of hepatic alveolar echinococcosis could be successfully assigned to one of the sonomorphological patterns based on the ultrasonographic classification scheme (EMUC) proposed in the present study.”* What is meant by EMUC??
The acronym for the classification has been described in the methods section: “As an acronym, we propose EMUC-US (Echinococcosis multilocularis Ulm classification - ultrasound).”