Reviewer's report

Title: practical guides for utilisation of ordinary leeches

Version: 1 Date: 23 February 2004

Reviewer: Joerg Graf

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General
1. The English in the manuscript needs to be improved. Already the title is not clear. The authors mention “practical guides”; do they mean practical guidelines? The authors use the term “ordinary leeches”, yet Hirudo medicinalis is the medicinal leech. If the authors suggest that ordinary leeches are not farm-raised or are purchased on markets instead of pharmacies, it should be stated specifically.

2. The title of this manuscript leads one to expect guidelines about the use of leeches, but most of the manuscript deals with other aspects, specifically the identification of bacteria associated with the leech and a hypochloric acid treatment. Given the title one expects a thorough review of the literature to come up with the best guidelines, but this was not done. The authors cite one such publication by Rao and Whitaker, 2003. Another recent one is Abdelgabar and Bhowmick, 2003, and there are several from the mid 90’s.

3. The information about the microbiota and its antibiotic sensitivity is not new. In addition, the identification of the bacteria does not appear to use the current nomenclature, see below. The hypochloric acid treatment is new, but not investigate thoroughly enough to make conclusions about its efficacy and issue guidelines. This is potentially important information but needs to be further investigated as suggested below.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Page 4, conclusion. It is not sufficient to “think” that the hypochloric acid solution prevents infections. The authors present no direct evidence to support this statement. See comment #5.  
2. Page 5. A lot of work has been done in the area of leech therapy and its associated infections and only three studies are cited. In addition, important reviews about Aeromonas as a pathogen such as the one by Janda and Abbot, 1998, are missing.

3. Page 6, Methods. The authors describe their sampling methods insufficiently. Did they test whether the betadine treatment kill all of the bacteria present on the skin of the leech? They described the use of cotton swabs to obtain samples from the digestive tract. How could they be sure that the bacteria were not from the 11 pairs of bladders that were shown to harbor bacteria by Busing et al., 1953? Did they collect samples from the crop or the intestine? How did the authors incubate the samples?  
4. Page 6. Many commercially available identification systems have been shown not to work well with Aeromonas, especially older systems as it seems to be in this case because they can not differentiate A. hydrophila and A. caviae and also report A. sobria instead of A. veronii bv sobria, see Janda and Abbott, 1998 and #7.

5. Page 7. The authors describe that hypocholoric acid at certain concentrations prevented bacterial growth because no colonies were recovered on agar plates. While the authors state that the water or mouth contained bacteria before the treatment is no quantitative information. By how many fold does this treatment reduce the bacterial load? Will the bacteria reappear? This is important information to assess the usefullness of this treatment.
6. Page 8. The authors report bacterial growth from the skin, intestine and water without quantification. How many bacteria were recovered from the different species? Quantitative information is important to assess the relative risk of an infection. For example, is there a million Aeromonas and one Pseudomonas on the mouth or are they in more similar proportions?

7. Page 8. The Aeromonadaceae family has currently 15 members. The authors use an outdated identification scheme as indicated by the identification of A. sobria which should be A. veronii biovar sobria. A. hydrophila and A. caviae can be easily separated by biochemical tests. Because these three species are the most important human pathogens of this genus, it is important to separate them. It is critical for the consistency in this research area to use the current nomenclature and identification schemes.

8. Page 10. The authors mention the “denaturation” of hemoglobin by the bacteria and describe the utilization of its products. Do they mean the hydrolysis? References should be given to support this.

9. Page 11. How do their results of species identification compare to other studies besides the one cited?

10. Page 12. The authors describe the use of hypochloric acid, how does this compare to the 0.02% chlorhexidine treatment suggested by Lucht et al.? The authors stated that the other treatment had no effect on the gut flora, but this study did not examine the effect of hypochloric acid on the gut flora, they only looked for an effect on the surface flora and the water.

11. Page 12. How does the hypochloric acid treatment affect the digestive tract bacteria?

12. Page 12. Is it known if the bacteria from the gut or from the skin cause the infection?

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. On page three the authors state: “we studied bacterial identification…” The authors did not study bacteria identification, they only reported their results.
2. Page 5. “In many countries, like us,” is unclear and grammatically incorrect. The authors should mention Turkey.
3. Page 6. Biomerieux is misspelled throughout the manuscript.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests: none