Author's response to reviews

Title: First case of infective endocarditis caused by Helicobacter cinaedi

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Author's response to reviews: see over
Revisions regarding the Case Report on Infective Endocarditis with *Helicobacter cinaedi* (MS: 1659590429136920)

Dear Editor

Thank you for considering our case report ‘First case of infective endocarditis caused by *Helicobacter cinaedi*’ for publication in *BMC Infectious Diseases*.

Please find below our answers to the comments made by the two reviewers:

**Reviewer 1**

Figure 1. Indicate the DNA concentrations and/or the amount of DNA used for PCR detection instead of the volume in µl. Indicate that the PCR 2 corresponds to a nested PCR.

*Answer: We measured the DNA concentration and indicated the DNA amount of the samples in Figure 1. As mentioned in the manuscript, PCR 2 corresponds to a normal single step PCR (lines 111-112,117-118)*

Figure 1 and results. Explain why the first PCR performed on DNA extracted from formalin-fixed aortic valve was negative. It could be related to the amount of *H. cinaedi* genome copies and/or to fragmented DNA.

*Answer: We strongly assume that the quality of the DNA from the formalin fixation, which leads to degradation and crosslinking of DNA, was responsible for the negative PCR of a product of a 659 bp. We added this explanation to the manuscript.*
Include a positive control in the PCRs (H. cinaedi DNA).

**Answer:** We ordered the reference strain DSM 5359 (corresponding ATCC 35683 and CCUG 18818) from the DSMZ strain collection. Unfortunately the strain cannot be sent timely because of technical problems. We therefore did not add a positive control to the figure.

Explain how urine samples could be positive for H. cinaedi DNA.

**Answer:** There are only little data on the pathophysiology of Helicobacter cinaedi infection. We assume that during this severe invasive infection DNA fragments may be excreted by the urogenital tract. For example, in Staphylococcus aureus bacteremia a bacteriuria is often observed.

PCR on serum sample could be performed.

**Answer:** To test serum samples would be an interesting issue. Unfortunately we had no access to serum samples from this patient retrospectively.

Minor essential revisions

Explain the rational of antimicrobial therapy. I agree that is there is guideline for the treatment of such infection. However the authors could refer to some publication (ex: Kiehlbauch JA, J Clin Microbiol 1995) in which they show that 19% of H. cinaedi clinical isolate can be resistant fo quinolones.

**Answer:** see answer for the question below

How ceftriaxone could be better than amoxicillin against H. cinaedi? This species is indeed naturally resistant to cephalotin.

**Answer:** As a matter of fact, we first recommended to complete therapy with amoxicillin, based on an epidemiologic study of 23 patients with H. cinaedi–associated illness (Ref. 4, Kiehlbauch et al.: Helicobacter cinaedi-associated bacteremia and cellulitis in immunocompromised patients. Ann Intern Med 1994, 121:90-93: “Many antimicrobial therapies were used to treat patients with H. cinaedi infection. From our series, it appears that treatment with a penicillin, tetracycline, or aminoglycoside may be more effective than treatment with cephalosporins, erythromycin, or ciprofloxacin.”). Unfortunately, the patient didn’t tolerate this therapy. Therefore, we had to change antibiotic treatment to ceftriaxone in combination with gentamicin. This regimen was chosen accordingly to a case of infective endocarditis (Ref 17, Hamada et al.: The first case of infective endocarditis caused by Helicobacter cinaedi identified by 16S rRNA gene sequencing and H. cinaedi specific-PCR. ID Week. San Francisco, CA; 2013) as well as accordingly to a case of recurrent bacteremia (Ref 5, Recurrent bacteremia with Helicobacter cinaedi: case report and review of the literature. BMC Infectious Diseases 2006, 6:86.) We added these information to the case description (lines 127-129) and completed the considerations with references in the discussion (lines 169-173).

Discretionary revision

Explain how DNA form paraffin embedded valve was extracted.
Answer: In line 115-117 we added the deparaffinization and DNA extraction method to the manuscript.

Reviewer 2

line 193 drafted rather than draftet.

Answer: was changed accordingly.

line 202 to 203 Should read: We thank Thomas J. Armstrong for revising the manuscript regarding the correct use of English.

Answer: was changed accordingly

Yours sincerely,

Veronika Baettig (in behalf of Daniel Goldenberger), MD

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