Author's response to reviews

Title: Frequent detection of Bocavirus DNA in German children with respiratory tract infections

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Response to reviewers and changes in the revised manuscript

Reviewer: Ian Mackay

Major revisions

* The number in the abstract has been corrected.
* A sentence pointing out that the true number of coinfections is probably higher than the observed 39.1 %, because several respiratory pathogens were not analyzed, has been added in the discussion (page 10).
* In general, seasonal differences may account for differences in infection frequencies. However, we share the opinion of the reviewer that this does not seem to be the case for hBoV infections in our study population. The paragraph on page 8 has been modified to clarify this issue and we hope that misunderstandings on this aspects will be avoided.
* To control for false positive amplification, general laboratory procedures to prevent PCR contamination were strictly adhered to and one negative control was extracted and amplified for every five NPA samples, as stated in the method section. In all runs, all negative controls were found to be negative for hBoV DNA. Following a single round PCR for detection of hBoV DNA, confirmation of the specificity of the PCR product other than by determination of the product length is necessary. This may be achieved, for example, by a nested PCR, hybridization, or sequence determination. Paragraph 4 on page 4 of the method section has been changed in order to avoid the impression that sequencing was meant to exclude contamination.

Minor and discretionary revisions

* HKU has been changed in HKU1
* Pg6, Para2: The percentage number has been corrected.
* Numbers and percentages have been provided in the result section.
* A statement regarding the different sensitivities of the PCR and IFA methods used in our study has been added on page 8, paragraph 1.
* All bocavirus studies, that were published when the paper was submitted for publication on 11 April, were included in the reference section and discussed in the text. Two additional reports (Bastien et al. from Canada, Foulongne et al. from France) appeared in the May issue of Emerging Infectious Diseases. These references have been included in the introduction, discussion and reference section of the revised version of the manuscript.
* Adeno has been changed to Adenovirus. The comma has been changed to a period.
* Parainfluenza has been changed to parainfluenzae.
* As also suggested by the reviewer Ron Fouchier, Table 4 has been changed to contain separate columns for children with and without coinfections. A difference of borderline significance was found for pneumonia. This aspect has been included in the result section (page 7) and the discussion (page 9).
* A footnote regarding the seasons of the observation periods was added in Tab. 2.

Discretionary revisions

* As suggested by the reviewer Ron Fouchier, the paper of TM. Rivers, J Bacteriol 1937, was added as
reference. The review of Fredricks and Relman is partly based on this reference.

* We tried to express the number of detections as a percentage in Figure 1. However, in our opinion clarity was lost because two different scales of the y-axis would have to be used. Therefore, we opted to leave the Figure 1 unchanged.

Reviewer: Ron Fouchier:

Minor revisions

1. "homology" has been replaced by "% identity of the nucleotide sequence"
2. Reference to the modified postulates of Rivers has been included.
3. On reanalysis, the limit of detection of our PCR assay was approximately 10 copies per reaction. This value is now provided on page 5 and 8.

Discretionary revisions

1. Table 4: See response to reviewer Ian Mackay.
2. A sentence pointing out that the true number of coinfections is probably higher than the observed 39.1 % has been added in the discussion (page 9).
3. We agree that the PCR assay employed in our study may detect positive samples with low viremia of children in the early or late infection phase. This aspect is currently being studied by a real-time PCR assay.

Reviewer: Mamdoh Meqdam:

Tab 4 has been revised to contain separate columns for children with and without coinfection. Because of low numbers, percentage data have been omitted in the revised table.