RESEARCH NOTE

The first genome sequences of human bocaviruses from Vietnam [version 2; peer review: 3 approved]

Tran Tan Thanh 1, Hoang Minh Tu Van 2, Nguyen Thi Thu Hong 1, Le Nguyen Truc Nhu 1, Nguyen To Anh 1, Ha Manh Tuan 2, Ho Van Hien 1, Nguyen Manh Tuong 1, Trinh Trung Kien 1, Truong Huu Khanh 3, Le Nguyen Thanh Nhan 3, Nguyen Thanh Hung 3, Nguyen Van Vinh Chau 4, Guy Thwaites 1,5, H. Rogier van Doorn 1,5, Le Van Tan 1

1 Oxford University Clinical Research Unit in partnership with the Hospital for Tropical Diseases, Ho Chi Minh, 700000, Vietnam
2 Children’s Hospital 2, Ho Chi Minh, 700000, Vietnam
3 Children’s Hospital 1, Ho Chi Minh, 700000, Vietnam
4 Hospital for Tropical Diseases, Ho Chi Minh, 700000, Vietnam
5 Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, OX3 7DQ, UK

Abstract

As part of an ongoing effort to generate complete genome sequences of hand, foot and mouth disease-causing enteroviruses directly from clinical specimens, two complete coding sequences and two partial genomic sequences of human bocavirus 1 (n=3) and 2 (n=1) were co-amplified and sequenced, representing the first genome sequences of human bocaviruses from Vietnam. The sequences may aid future study aiming at understanding the evolution of the virus.

Keywords

human bocavirus, HBoV, HFMD, Vietnam

This article is included in the Oxford University Clinical Research Unit (OUCRU) gateway.

Open Peer Review

Reviewer Status

Invited Reviewers

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>✅</td>
<td>✅</td>
<td>✅</td>
</tr>
</tbody>
</table>

version 2
published 09 Jan 2017

version 1
published 16 Nov 2016

1 Bas B. Oude Munnink, Erasmus MC, Rotterdam, The Netherlands
2 Verena Schildgen, Hospital of University of Witten/Herdecke, Cologne, Germany
   Oliver Schildgen, gGmbH Clinic of Cologne, Hospital of University of Witten/Herdecke, Cologne, Germany
3 Xiu-ling Ji, Kunming University of Science and Technology, Kunming, China

Any reports and responses or comments on the article can be found at the end of the article.
**Corresponding author:** Le Van Tan (tanlv@oucru.org)

**Competing interests:** No competing interests were disclosed.

**Grant information:** This work was supported by the Wellcome Trust [101104/Z/13/Z], [106680/B/14/Z]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Copyright:** © 2017 Thanh TT et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**How to cite this article:** Thanh TT, Van HMT, Hong NTT et al. The first genome sequences of human bocaviruses from Vietnam [version 2; peer review: 3 approved] Wellcome Open Research 2017, 1:16 (https://doi.org/10.12688/wellcomeopenres.10042.2)

**First published:** 16 Nov 2016, 1:16 (https://doi.org/10.12688/wellcomeopenres.10042.1)
Amendments from Version 1

In this revised version, we have 1) included a brief description of our MiSeq sequencing approach, 2) provided a table (Table 1) summarizing demographic, respiratory/gastrointestinal signs/symptoms, and virological information of the four patients in whom HBoV was detected, and 3) included a discussion covering general issues related to our report as per reviewers’ suggestions.

See referee reports

Introduction

Human bocaviruses (HBoV) are non-enveloped, single stranded DNA viruses of the family Parvoviridae, subfamily Parvovirinae and genus Bocaparvovirus. The virus genome is ~5.3 Kp in length. HBoV-1 was first discovered in 2005. Since then three additional HBoV species, namely HBoV-2, HBoV-3 and HBoV-4, have been discovered. While the clinical significance of HBoV remains unknown, worldwide their prevalence in respiratory/gastrointestinal tracts varies between 0–26%. In Vietnam, the reported prevalence of HBoV was 2–17%. Currently, there is relatively limited sequence information, especially at genome-wide level, of HBoV from Vietnam, although such knowledge may be essential for the development of sensitive, specific diagnostic PCR for the local viral strains, and may aid future investigation documenting the circulation and spread of the viruses at global scale.

Herein we report the recovery of two complete coding sequences (CDS) and two partial genomic sequences of HBoV from swabs of Vietnamese children enrolled in our ongoing hand, foot and mouth disease (HFMD) research program in Ho Chi Minh City. The research program aims to look at various disease aspects, including pathogen evolution and its potential implication for vaccine development and implementation.

Methods and results

Whole-genome sequencing of the dominant pathogens (including coxsackievirus A6 (CV-A6), CV-A10 and CV-A16) were performed on 296 RT-PCR positive swabs using an in-house MiSeq-based approach. In brief, 110 µl of selected swabs were centrifuged at 13,500 rpm for 10 minutes to remove host cells or large cellular components. After DNase treatment, viral nucleic acid (NA) was then isolated from 100 µl of supernatant using QIAamp viral RNA kit (QIAgen GmbH, Hilden, Germany), and recovered in 50 µl of elution buffer (provided with the kit). Ten microliter of the isolated NA was subjected to cDNA synthesis using Super Script III kit (Invitrogen, Carlsbad, CA, USA) and FR26RV-Endoh primer (primer sequences can be found elsewhere). The cDNA was then converted to double-stranded DNA using exon-Klenow (Invitrogen), and subsequently pre-amplified using Platinum PCR supermix (Invitrogen) and FR20RV primer. PCR product was then purified and subjected to library preparation using Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA) and was finally sequenced using MiSeq reagent kits (Illumina) in an Illumina MiSeq platform (Illumina).

After reference-based mapping to generate the complete genome sequences of the targeted enteroviruses using Geneious software v 8.1.5 (Biomatters, Ltd, Auckland, New Zealand), the remaining reads were then subjected to publicly available metagenomic pipelines; Taxonomer and Sequence-based Ultra-Rapid Pathogen Identification (SURPI) to explore the contents of non-enteroviral sequences in the tested swabs. Evidence of bocavirus sequences were found in four swabs (including 3 throat- and 1 rectal swabs). A reference-based mapping approach using Geneious software (Biomatters) was then employed to recover the HBoV genomes from the corresponding dataset. Subsequently, 2 CDS (1 from a throat swab with 4925 bp in length and the other from a rectal swab with 4898 bp; i.e. over 90% of genome coverage) were successfully assembled with a mean coverage of 1,922 and 3,745, respectively. In the other two datasets from the remaining 2 swabs only partial genomic sequences of HBoV, each with 2870 bp in length and a mean coverage of 15.4 and 448.7, were recovered.

Subsequent sequence alignment and phylogenetic analysis using MUSCLE and Neighbor-joining available in Geneious (Biomatters), respectively (Figure 1) revealed that all 3 Vietnamese HBoV recovered from the throat swabs belonged to HBoV-1 and had >98% of sequence similarity at nucleotide level with other HBoV-1. The other belonged to HBoV-2 and had a close relatedness with...
Figure 1. Phylogenetic trees showing the relationship between the Vietnamese bocaviruses and representative worldwide circulating strains. A) Neighbor-joining phylogeny of CDS; B) Neighbor-joining phylogeny of partial genomic sequences spanning the region from nucleotide 1897 to 4856 of the HBoV genomes; C) Neighbor-joining phylogeny of ORF1 encoding NS1 protein; D) Neighbor-joining phylogeny of ORF2 encoding NP1 protein; E) Neighbor-joining phylogeny of ORF3 encoding VP1 and VP2 proteins. Trees were reconstructed using Neighbor-joining method available in Geneious with Tamura-Nei nucleotide substitution model, and support for individual nodes was assessed using a bootstrap procedure (1000 replicates). Bootstrap values greater than 60% are shown on the branch nodes. The Vietnamese strains from this study are indicated by solid triangles. The scale bars indicate the number of nucleotide substitution.

A Thai strain CU54TH (GU048663) with a sequence similarity of 97.3% (Figure 1). Similar results were obtained when the analyses were done for 3 individual open reading frames (ORF1, ORF2, and ORF3) of the virus genome (Figure 1).

All the four HFMD patients (including 3 CV-A6 and 1 CV-A12, Table 1) in whom HBoV was detected had mild HFMD, and were enrolled in November 2013 – March 2014. Three had vomiting, and two presented with runny nose and cough (Table 1).
<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (year)</th>
<th>Admission date</th>
<th>HFMD grade</th>
<th>Respiratory/gastrointestinal signs/symptoms</th>
<th>Sample</th>
<th>Enterovirus</th>
<th>Bocavirus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Runny nose, Cough, Vomiting, Diarrhea</td>
<td></td>
<td>Serotype</td>
<td>Species</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Throat swab</td>
<td></td>
<td>Full genome</td>
<td>Complete CDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean coverage</td>
<td>Mean Coverage</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>0.9</td>
<td>11/3/2014</td>
<td>2a</td>
<td>Yes, Yes, Yes, No</td>
<td>Throat swab</td>
<td>CV-A6</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6,024</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,922</td>
<td>KY050743</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>0.4</td>
<td>5/11/2013</td>
<td>2a</td>
<td>No, No, No, No</td>
<td>Rectal swab</td>
<td>CV-A6</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12,303</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3,745</td>
<td>KY050744</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>1.4</td>
<td>14/11/2013</td>
<td>1</td>
<td>No, No, Yes, No</td>
<td>Throat swab</td>
<td>CV-A12</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2,841</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.4</td>
<td>KY072816</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>0.8</td>
<td>4/12/2013</td>
<td>1</td>
<td>Yes, Yes, Yes, No</td>
<td>Throat swab</td>
<td>CV-A6</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2,493</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>448.7</td>
<td>KY072817</td>
</tr>
</tbody>
</table>

**Table 1.** Demographic, clinical information, and details of MiSeq sequencing results.
Discussion
Herein we reported for the first time 2 complete CDS alongside two other partial genomics sequences of HBoV from Vietnam. Phylogenetically, the four HBoVs from Vietnam were closely related to other HBoV strains sampled from various countries worldwide, reflecting a wide distribution of these HBoV lineages at global scales.

All three HBoV detected in throat swabs belong to species 1, while the remaining virus detected in rectal swab was HBoV-2. This is in line with previous reports regarding the frequent detection of HBoV-1 and HBoV-2 in throat- and rectal swab, respectively.\textsuperscript{5,6,11-18} Albeit our sample size was small. Likewise, all the four HFMD patients in whom HBoVs were found were enrolled into our HFMD study during the seasonal peak of HBoV in southern Vietnam.\textsuperscript{19}

Although the pathogenic potential of HBoV infections remains unknown, clinical signs/symptoms such as vomiting, runny nose and cough were also commonly recorded among HFMD patients in previous reports.\textsuperscript{22} HBoV has commonly been co-detected with other pathogens in respiratory and gastrointestinal tracts.\textsuperscript{5,6,11-18} It was also previously detected in fecal samples of HFMD patients from Thailand.\textsuperscript{22} Clearly, further research is needed to ascribe the contribution of coinfections to clinical manifestation and pathogenesis of HFMD. Of note, previous reports showed that there might be an association between coinfections with other viral pathogens such as norovirus and rotavirus and clinical severity of HFMD patients.\textsuperscript{21}

In conclusion, to the best of our knowledge, we are the first to report the complete CDS of HBoVs from Vietnam. The contribution of HBoV to clinical manifestation of HFMD requires further research.

Data availability
Nucleotide sequence accession numbers: the HBoV sequences have been submitted to Genbank (https://www.ncbi.nlm.nih.gov/\texttt{genbank/}) under accession numbers KY050743, KY050744, KY072816 and KY072817.

Consent
The clinical samples used in this study were derived from an on-going HFMD study in three referral hospitals in Ho Chi Minh City, Vietnam. The study was reviewed and approved by the local Institutional Review Boards and the Oxford Tropical Research Ethics Committee (OxTREC), University of Oxford, Oxford, United Kingdom. Written informed consent was obtained from parent or legal guardian of each participant.

Author contributions
TTT and LVT: designed the study, analysed the test results, and drafted the manuscript. HMTV, NTTH, LNTN, NTA, HMT, HVH, NMT, TTK, THK, LNTN, NTH, NVVC, GT, and RHvD: enrolled patients, took samples and did laboratory testing. All authors have read the final manuscript and agreed with its contents.

Competing interests
No competing interests were disclosed.

Grant information
This work was supported by the Wellcome Trust [101104/Z/13/Z], [106680/B/14/Z].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We thank Ms Le Kim Thanh from Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam for her logistic assistance and Truong Duy for his help with the resolution of the figure of the phylogenetic trees. We are indebted to patients and their parents for their participation in this study, and all the nursing and medical staff at the at Children’s Hospital 1, Children’s Hospital 2 and the Hospital for Tropical Diseases who provided care for the patients and helped collect clinical data.

References
9. Tran DN, Trinh QD, Pham NT, et al.: Clinical and epidemiological characteristics of...


Open Peer Review

Current Peer Review Status: ✔ ✔ ✔

Version 2

Reviewer Report 13 January 2017
https://doi.org/10.21956/wellcomeopenres.11425.r19091

© 2017 Schildgen V et al. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Verena Schildgen
Department of Pathology, gGmbH Clinic of Cologne, Hospital of University of Witten/Herdecke, Cologne, Germany
Oliver Schildgen
Department of Pathology, gGmbH Clinic of Cologne, Hospital of University of Witten/Herdecke, Cologne, Germany

All previous comments have been thoroughly addressed and the manuscript has significantly improved.

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 12 January 2017
https://doi.org/10.21956/wellcomeopenres.11425.r19090

© 2017 Oude Munnink B. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Bas B. Oude Munnink
Viroscience, Erasmus MC, Rotterdam, The Netherlands

The authors have carefully addressed all comments raised in the previous review.

Competing Interests: No competing interests were disclosed.
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 11 January 2017

https://doi.org/10.21956/wellcomeopenres.11425.r19089

© 2017 Ji X. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Xiu-ling Ji
Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, China

I suggest this manuscript can be indexed.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 05 December 2016

https://doi.org/10.21956/wellcomeopenres.10819.r18202

© 2016 Ji X. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Xiu-ling Ji
Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, China

Human bocavirus (HBoV) was first identified in 2005, and regarded as a causative pathogen of respiratory tract diseases. The paper reported the recovery of two complete coding sequences and two partial genomic sequences of HBoV from swabs of Vietnamese children enrolled in HFMD research program in Ho Chi Minh City. The experiments were well designed and performed. In addition, the results were described almost properly. In this meaning, the manuscript is sound and suitable for the indexing. However, as I mentioned below, this manuscript has still some points to be clearly explained.

1. Comparison of the genome sequences of human bocaviruses between from Vietnam and the others should be much more discussed.
2. To date, all of the HBoV genotypes contain the episomal structure. Then, it would be better to analyze it in the paper.

3. The genome of HBoV is organized in three ORFs: ORF1 encoding NS1 protein; ORF2 encoding NP1 protein; ORF3 encoding VP1 and VP2 proteins. So it was suggested that the Phylogenetic trees of nucleotide and amino acid sequences of the HBoV genes should be constructed.

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Jan 2017
Thanh Tran Tan, Oxford University Clinical Research Unit in partnership with the Hospital for Tropical Diseases, Ho Chi Minh, Vietnam

1. Comparison of the genome sequences of human bocaviruses between from Vietnam and the others should be much more discussed.

   **Response:**
   We have now discussed this in the discussion section. The second sentence of the discussion reads “Phylogenetically, the four HBoVs from Vietnam were closely related to other HBoV strains sampled from various countries worldwide, reflecting a wide distribution of these HBoV lineages at global scales”.

2. To date, all of the HBoV genotypes contain the episomal structure. Then, it would be better to analyze it in the paper.

   **Response:**
   We thank the referee for this comment. Please forgive our ignorance but we understood that episomal structure is formed by repeated sequences at the 5’ and 3’ ends, which unfortunately were not fully sequenced. Therefore the analysis could not be done reliably.

3. The genome of HBoV is organized in three ORFs: ORF1 encoding NS1 protein; ORF2 encoding NP1 protein; ORF3 encoding VP1 and VP2 proteins. So it was suggested that the Phylogenetic trees of nucleotide and amino acid sequences of the HBoV genes should be constructed.

   **Response:**
   We have reconstructed additional phylogenetic trees according to the suggestion of Dr Xiu-ling Ji. We have therefore added those additional phylogenetic to this revised version, and added a sentence to elaborate it in the result section; “Similar results were obtained when the analyses were done for 3 individual open reading frames, ORF1, ORF2 and ORF3 (Figure 1)”.

**Competing Interests:** No competing interests were disclosed.
Verena Schildgen
Department of Pathology, gGmbH Clinic of Cologne, Hospital of University of Witten/Herdecke, Cologne, Germany

Oliver Schildgen
Department of Pathology, gGmbH Clinic of Cologne, Hospital of University of Witten/Herdecke, Cologne, Germany

Although the article adds novel information on HBoV epidemiology in Vietnam and presents the sequences of current strains in this geographic region, it has numerous technical shortcomings. The methodology used is not sufficiently described. E.g., the authors state that coxsackieviruses were detected by whole genome sequencing. This technique, however, would detect genomic host DNA, coxsackie is an RNA virus. Moreover, primer sequences and PCR protocols are missing, and alignments are not shown. The reference list is extremely short, and the overall description of methods, results and discussion is weak.

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Thanh Tran Tan, Oxford University Clinical Research Unit in partnership with the Hospital for Tropical Diseases, Ho Chi Minh, Vietnam

Response:
We thank Dr Verena Schildgen and Dr Oliver Schildgen for their constructive comments. Please allow us to clarify that the method used was developed in our laboratory for amplification and sequencing viral sequences, and it has been published (citation #11). Although host DNA can also be simultaneously sequenced, investigation of its presence in the obtained reads is beyond the scope of the present Research Note.
Given the method used was detailed in our previous publication (including primer sequences), we have chosen to briefly present it in this revised version as per the reviewers' comment. Likewise, we have updated our reference list (from 11 to 23 references), and added more text to the discussion section. Please also refer to our responses to the other reviewers regarding the updated result section, while we hope the reviewers appreciate that the format of a Research Note is concise.
This paper describes the first complete genome sequences of human bocaviruses in Vietnam. Reporting complete genome sequences from potential local viral pathogens is vital to develop accurate diagnostic methods and to perform additional studies. However, depending on the scope of this journal, this paper would also be suitable for publication in the journal Genome Announcements.

The paper is very compact and carefully written, however I feel that some essential information is missing:

- The authors show that they have detected 4 bocaviruses in enterovirus positive samples, as identified by RT-PCR, which demonstrates the strength of agnostic deep sequencing. However, could it be that the symptoms observed in these patients was caused by these enteroviruses and not by the bocaviruses detected in these samples? And which specific enteroviruses (or other viral pathogens) were detected in these bocavirus positive samples?

- Three viruses were found in throat swabs while one virus was found in rectal swabs. Which virus was found in which sample? E.g. was the genome coverage in the rectal swab lower and does this perhaps also explain the different species detected — was species 2 found in rectal swabs and species 1 in throat swabs?

And some minor points:

- In the abstract the authors mention that “The sequences may aid future study aiming at understanding the evolution of the pathogen”. However, in the introduction and conclusion they mention that the clinical significance of bocavirus infection remains unknown. I suggest to change the word pathogen by virus.

- The phylogenetic tree is difficult to read in the current resolution

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Author Response 03 Jan 2017**

Thanh Tran Tan, Oxford University Clinical Research Unit in partnership with the Hospital for Tropical Diseases, Ho Chi Minh, Vietnam
1. The authors show that they have detected 4 bocaviruses in enterovirus positive samples, as identified by RT-PCR, which demonstrates the strength of agnostic deep sequencing. However, could it be that the symptoms observed in these patients was caused by these enteroviruses and not by the bocaviruses detected in these samples? And which specific enteroviruses (or other viral pathogens) were detected in these bocavirus positive samples?

Response:
We agree with the referee that the observed signs/symptoms might have been caused by HFMD causing enteroviruses, and have discussed this in the discussion section added to this revised version.

We have provided the information about the specific enteroviruses detected in the four HFMD in the text and Table 1 added to this revised version. The second sentence from the last of the result section now reads “All the four HFMD patients (including 3 CV-A6 and 1 CV-A12, Table 1) in whom HBoV was detected had mild HFMD, and were enrolled in November 2013 – March 2014.”

2. Three viruses were found in throat swabs while one virus was found in rectal swabs. Which virus was found in which sample? E.g. was the genome coverage in the rectal swab lower and does this perhaps also explain the different species detected — was species 2 found in rectal swabs and species 1 in throat swabs?

Response:
We found HBoV-1 in 3 throat swabs and HBoV-2 in 1 rectal swab. There was no correlation between genome coverage and sample types (i.e. rectal/throat swab), although the sample size was small. We have presented those data in the original manuscript and have now modified the text slightly to further elucidate the referee’s comment and provided the details in Table 1.

“Evidence of bocavirus sequences were found in four swabs (including 3 throat- and 1 rectal swabs). A reference-based mapping approach using Geneious software (Biomatters) was then employed to recover the HBoV genomes from the corresponding dataset. Subsequently, 2 CDS (1 from a throat swab with 4925 bp in length and the other from a rectal swab with 4898bp; i.e. over 90% of genome coverage) were successfully assembled with a mean coverage of 1,922 and 3,745, respectively (Table 1). In the other datasets from the remaining two swabs only partial genomic sequences of HBoV, each with 2870bp in length and a mean coverage of 15.4 and 448.7, were recovered.

Subsequent sequence alignment and phylogenetic analysis using MUSCLE and Neighbor-joining available in Geneious (Biomatters), respectively (Figure 1) revealed that all 3 Vietnamese HBoV recovered from the throat swabs belonged to HBoV-1 and had >98% of sequence similarity at nucleotide level with other HBoV-1. The other recovered from rectal swab belonged to HBoV-2 and had a close relatedness with a Thai strain CU54TH (GU048663) with a sequence similarity of 97.3% (Figure 1).”

Additionally, we have also discussed the detection of HBoV-1 and HBoV-2 in throat- and rectal swab, respectively in the discussion section.
3. In the abstract the authors mention that “The sequences may aid future study aiming at understanding the evolution of the pathogen”. However, in the introduction and conclusion they mention that the clinical significance of bocavirus infection remains unknown. I suggest to change the word pathogen by virus.

Response:
We have replaced the word “pathogen” by “virus” as recommended.

4. The phylogenetic tree is difficult to read in the current resolution

Response:
We have improved the resolution of the phylogenetic trees.

**Competing Interests:** No competing interests were disclosed.