Author’s response to reviews

Title: Deposition of respiratory virus pathogens on frequently touched surfaces at airports

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Version: 1 Date: 25 Apr 2018

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Responses to reviewer comments

25th of April, 2018

INFD-D-18-00431

Deposition of respiratory virus pathogens on frequently touched surfaces at airports Niina Ikonen, MSc; Carita Savolainen-Kopra, PhD; Joanne E. Enstone, PhD; Ilpo Kulmala, PhD; Pertti Pasanen, PhD; Anniina Salmela, MSc; Satu Salo, PhD; Jonathan S. Nguyen-Van-Tam, PhD; Petri Ruutu, PhD BMC Infectious Diseases
Dear Dr Hassan Zaraket,

the Editor of BMC Infectious Diseases

Thank you for opportunity to revise our manuscript. We have reviewed the comments from both reviewers and based on those revised our manuscript. Please find below our point-by-point response. The changes in the manuscript are made with track changes mode.

We hope that you find our revised manuscript suitable for publication in BMC Infectious Diseases.

Yours sincerely,

Niina Ikonen

Point-by-point responses to reviewers’ comments

Reviewer 1 (Julian Wei Tze Tang, BA MBChB MA PhD MRCP FRCPath FHKCPath FHKAM):

The authors cover most of the limitations, including the relatively low number of samples, but why did they take so few air-samples?

- Preliminary sampling in September 2015 showed that the amount of bacteria collected on filter from air was relatively low. Based on that information the focus was not on air samples but on
identifying so called hot spots and sites with residues of microbes. Air sampling is methodologically challenging. Air sampling needs to be developed further by collecting bigger volumes, concentrating microbes from filter, selection of filter type etc.

As the authors stated, the interpretation of the results is difficult because we don't know how long those viruses have been on those surfaces before the swabs were taken – did they authors consider any viral culture/viability studies? This is becoming the norm for these environmental studies now.

If not, any reasons why? The relatively high CT (so low viral load) values?

- In this study a primary aim was to identify so called hot spots, ie sites where the risk for microbe transmission is expected at least periodically to be increased due to favorable conditions or human behavior, and sites with residues of microbes and guide measures to minimize transmission for example during an emerging pandemic threat or severe epidemic.

The authors did not consider viral culture because it is not as sensitive a method as PCR. It is also known that some viruses such as some coronaviruses and species C rhinoviruses cannot be cultivated using traditional cell culture methods.

There is no revision in the manuscript.

Some analysis of security camera footage recording/describing and analysing passenger touch frequency of these sampled surfaces would have been useful to identify the risk that such potentially contaminated surfaces pose to passengers - the authors could propose this for future studies.
Thank you for this comment. It is a good idea, but the use of security camera for this purpose would cause problems related to the privacy of the individuals recorded. On the other hand the use of volunteers consenting to recording could cause bias in the study.

Based on our study results we are considering this approach focusing especially on security check boxes in future studies in other airports. By marking boxes you could count how many people have used the box between samplings.

Finally, I would like the authors to include more details (including the limit of detection - LOD) of their detection PCR assay - including the primer/probe sequences, as the purpose of a paper is to allow other teams to accurately reproduce their assay/study elsewhere.

The Methods section, the paragraph Nucleic acid extraction and virus detection, page 5 has been revised. Nucleic acid extraction and virus detection are described in more detail including also the references for primer and probe sequences.

Also, did the authors attempt to sequence any of the POS virus samples - to further characterise them epidemiologically in a local, national or global context? This would add more depth and weight to the paper - though the CT values are probably too high for this to have been successful, admittedly - but this is typical for environmental samples.

In our standard respiratory virus surveillance we use sequencing to characterise virus strains (genetic characterisation) only for influenza viruses. It is not a standard procedure for other respiratory viruses. In this study we tried to subtype the influenza A positive specimen by sequencing and by real-time PCR but we did not succeed. If we had succeeded to multiply the hemagglutinin gene, it would have given the opportunity to compare the sequence of the hemagglutinin with HA sequences from different parts of the world and for possibly providing us with the information of the origin of the viral strain and its epidemiological context.
We have revised the manuscript accordingly and the added sentence can be found at Discussion section, the last sentence in the last paragraph, page 7.

The authors should state these additional limitations in their Discussion if these additional steps were not attempted.

- The authors have made modifications at the Discussion section, the fifth paragraph, page 7. The other limitations of the study are mentioned at the sixth paragraph.

Reviewer 2 (John Lednicky, Ph.D.):

The PCR-based tests for the different viruses could be mentioned: Either provide references for the tests or if the tests were designed by the authors, tell the reader which primers were used and the targets of the primers.

- The Methods section, the paragraph Nucleic acid extraction and virus detection, page 5 has been revised. Nucleic acid extraction and virus detection are described in more detail including also the references for primer and probe sequences.

The authors should mention why they did not attempt virus isolation. Had viruses been isolated in cell cultures, one could make a stronger argument that infectious viruses were present on high-touch surfaces and thus pose a potential risk for self-inoculation and subsequent infection. The infectious dose is difficult to measure when it comes to viruses deposited on environmental surfaces; the authors are thus correct in stating it is not a simple task to correlate virus quantity on a high-touch area, quantity of virus picked up by touch of the contaminated area, and amount of virus that gets self-inoculated and leads to illness. By airborne routes, some viruses including human influenza A viruses can cause infection at very low delivered doses (example: one to five
infectious virus particles when inhaled can cause an infection in humans). Nevertheless, if the viruses on the environmental surfaces are non-viable. There do not pose a biohazard.

- Thank you for the comment. The authors agree with the reviewer. In this study a primary aim was to identify so called hot spots, ie sites where the risk for microbe transmission is expected at least periodically to be increased due to favorable conditions or human behavior, and places with residues of microbes and guide measures to minimize transmission for example during an emerging pandemic threat or severe epidemic.

The authors did not consider viral culture because it is not as sensitive a method as PCR. It is also known that some viruses such as some coronaviruses and rhinovirus C cannot be cultivated using traditional cell culture methods.

The authors agree that if the viruses on environmental surfaces are not viable, they do not pose a hazard. However as PCR is unable to distinguish between viable and unviable, we cannot assume they are not viable, thus we conclude that we cannot exclude indirect contact transmission. There is no revision in the manuscript.

Readers of this paper will probably assume that to keep the experiment focused, and to reduce overall costs, the viruses chosen for detection were limited to those listed on page 5 (lines 14 to 16). Is that the case?

- Yes. We decided to use our standard respiratory panel that we currently use for our respiratory virus surveillance.

Which adenoviruses are detected by the PCR assays? Since adenoviruses are DNA viruses, the statement made in line 14, page 5 (reverse transcriptase PCR) is of course not correct for those viruses.
- Primers and probe were published by Damen et al, 2008, J Clin Microbiol 46 (12): 3997-4003.

The real-time PCR assay has been reported to detect all known human adenoviruses. Since the publication was eight years old at the time the study was done it is possible that some new adenovirus variants will not be detected with these primers and probe.

The statement concerning adenovirus and reverse transcriptase PCR is not correct as the reviewer has observed. We have taken note on it and modified the manuscript text.

Do the tests used by the authors detect rhinovirus C?


Do the tests used by the authors discriminate RSV types?

- No. The test is only used for detection of RSV positive specimens.

Finally, did the authors consider sequencing the virus genomes to confirm ID and also reveal which virus strains were in circulation?

- In our standard respiratory virus surveillance we used sequencing to characterise virus strains (genetic characterisation) only for influenza viruses. It is not a standard procedure for other respiratory viruses. We tried to subtype the influenza A positive specimen by sequencing and by real-time PCR but we did not succeed.

We have mentioned this in the manuscript text at Discussion section, last sentence in the last paragraph, page 7.
Was there a reason paramyxoviruses such as metapneumovirus and parainfluenza viruses were not tested for? And what about echo-, entero-, coxsackie -, and related viruses?

- We decided to use our standard respiratory panel that we currently use for our respiratory virus surveillance and this way to get known how effective were the sampling and recovery techniques.