## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Zinc for the prevention or treatment of acute viral respiratory tract infections in adults: a rapid systematic review and meta-analysis of randomised controlled trials. |
| **AUTHORS** | Hunter, Jennifer; Arentz, Susan; Goldenberg, Joshua; Yang, Guoyan; Beardsley, Jennifer; Myers, Stephen; Mertz, Dominik; Leeder, Stephen |

## VERSION 1 – REVIEW

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| **REVIEWER** | Moore, Michael<br>University of Southampton Medical School, Primary Care Medical Group |
| **REVIEW RETURNED** | 26-Jan-2021 |

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| **GENERAL COMMENTS** | BMJ Open review zinc for RTI<br><br>This is a new review examining the role of zinc administered by various routes in the prevention and treatment of acute RTI symptoms. It forms part of a review targeted at the role of zinc in the treatment and prevention of Covid-19.<br><br>The review is well written and clear.<br><br>The background seems appropriate.<br><br>The methods are well described and the protocol changes are detailed in the first paragraph.<br><br>The results section was well structured but I found the section on prevention studies and the differential effects according to illness severity hard to follow.<br><br>The findings regarding symptom severity are inconsistent with the effect on 3 day severity reaching clinical significance where-as the effect on average daily symptom scores was non-significant. The clinical significance of the 3 day score was only agreed post hoc and this might be considered in the limitations.<br><br>Abstract |

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| | The abstract makes no mention that this paper is presenting the secondary outcomes for a review focussed on the role of zinc in ameliorating Covid-19 infection. The rapid review has been adapted from that originally described in the Prospero registration including changing limits to some searches. This should be made clear at some point in the abstract. The same explanation should be included in the limitations section in the discussion.<br><br>The third bullet point in strengths and limitations is missing some text<br><br>Discussion.<br><br>The findings are appropriately summarised. Again I find this sentence (line 8) hard to follow which relates to the differential findings for illness severity This risk was substantially lower for preventing more severe symptoms such as fever.<br><br>The implications for clinicians may be overstated given the uncertainty over effects on acute illness severity/duration. These uncertainties are explored in the subsequent paragraph.<br><br>The concluding paragraph is appropriately worded.<br><br>Figure 4 was hard to read |

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| **REVIEWER** | Wilson, Peter<br>University College London Hospitals NHS Foundation Trust, Microbiology |
| **REVIEW RETURNED** | 26-Feb-2021 |

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| **GENERAL COMMENTS** | This is a systematic review and meta-analysis in an area of potential interest during the pandemic. There was an increase in mild adverse effects but there was a reduction in viral respiratory infection or the symptoms. The review follows Cochrane guidance and PRISMA and involved patient representatives. The databases searched were wide ranging. Data review was not by two reviewers in all cases, but calibration was used between reviewers. GRADE was used in assessing evidence but a quality index was not.<br>The results seem sound and conclusive in respective of a preventative effect and a reduction in symptoms of infection. The review makes a contribution to knowledge beyond earlier reviews in the scope of papers covered. Anosmia as an adverse effect is clearly of concern during the Covid-19 pandemic as diagnosis will be compromised and should be discussed. The use of a single albeit calibrated reviewer does carry a small risk of error. The conclusions appear to be justified and the use of zinc may be a useful preventive measure in some patients. However, it would be helpful to have some practical recommendations for future practice, particularly as this may be self-selected and applied by the public without medical intervention. |

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| | The protocol provided reflects the original plan to examine coronavirus rather than the revised version submitted which includes all viral respiratory infections. This should be clarified. BMJ Open published all the methods in 2020. Full documentation has been provided for search strategies, excluded and included references. |

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| **REVIEWER** | Singh, Surjit<br>AIIMS Jodphur, Pharmacology |
| **REVIEW RETURNED** | 13-May-2021 |

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| **GENERAL COMMENTS** | A Very well written study. A timely done study for facilitating the use of Zinc for COVID-19.<br><br>Risk of bias<br>It is usually understood that risk of bias for adverse effects should be low risk of bias even it is subjective. As adverse events are reported by patients, less likelihood of bias.<br>I was not able to understand the following differences in ROB in adverse events:<br>Eby 1984 – Why it is some concerns with randomization despite low ROB in duration and severity. According to me it is not possible.<br>There are some differences in ROB assessed with regard to adverse events and other efficacy outcomes. This is quiet unlikely to happen as it is rare to have missed data for AE, issues with randomization or blinding if it is not there for efficacy outcomes.<br>Results<br>Figure 2: Kindly check the data of Wei 2009 and Zhang 2009 as this data only has shown significant result because of which we have evidence of efficacy. I was unable to retrieve the articles. If you have kindly upload them next time so that I can check the data of Community acquired mild to moderate RTI.<br>I do not completely agree with the GRADE of moderate quality with regard to this outcome. No publication bias assumed does not apply to this outcome as only four studies were reporting the data. Hence there is publication bias which will downgrade the evidence to low rather than moderate. Kindly discuss with colleagues and some other experts in systematic reviews.<br>No publication as assessed by Eggers regression test is of outcomes which were reported by majority of studies.<br>Serious Adverse events – It should be mentioned that studies did not report the SAE if they did not have mentioned it. If studies have mentioned that there are no serious AE only then the conclusion drawn by authors is correct. Kindly check it.<br>In addition if no SAE were reported then ROB should be high as all the studies data were included. Therefore, overall Low evidence quality for SAE.<br>Appendix: Meta-analysis results (4.1) on page 6.<br>Mean duration of symptoms are positive days while 95% CI is negative or decrease in days. Kindly check.<br>Funnel plots Figure – All three figures: Visual inspection is suggestive of asymmetry. However Egger's regression test or Harbord score showed that there is no publication bias. This should be your conclusion.<br>Page 108 – 6.5 – Days taken for improvement<br>Data of Mossad 2003. Medians are taken as means as per Cochrane. Cochrane Handbook said that SD should be calculated as IOR/1.35 which comes out to be 2.2 in zinc and 2.59 in control group instead of 0.75 and 0.88 as mentioned by you. I have |

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| | calculated it from article of Mossad et al 2003. Kindly enter the correct data for synthesis of plots.<br>GRADE<br>Overall moderate quality of evidence for acute viral RTI and SAE should be checked and discussed as both seemed to be of low quality. |

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| **REVIEWER** | Liang, Chun-Yu<br>National Defense Medical Center, School of Nursing |
| **REVIEW RETURNED** | 10-Jun-2021 |

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| **GENERAL COMMENTS** | Overall, this is a clear, concise, meaningful, and well-written manuscript. There are few comments as followed.<br>1. According to PRISMA 2020 guideline, it is appropriate to measured consistency using Cochran Q text and $I^2$ in each subgroup analysis.<br>2. p7, it is suggested to add the statistical methods using in meta-regression for the outcome of RTIs.<br>3. Appendix 4, the p-value= 0.000 should change to p-value < .001 in tables.<br>4. p94, please explains more about " When no RTIs were reported in one study arm, 0.5 was recorded to facilitate analysis." Why used the number of 0.5 and how to avoid bias?<br>5. p 100 figure 1, please show the result of Egger's regression for the test of bias. |

## VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Michael Moore, University of Southampton Medical School Comments to the Author:

BMJ Open review zinc for RTI
This is a new review examining the role of zinc administered by various routes in the prevention and treatment of acute RTI symptoms. It forms part of a review targeted at the role of zinc in the treatment and prevention of Covid-19. The review is well written and clear.
The background seems appropriate.
The methods are well described and the protocol changes are detailed in the first paragraph.

**1.** The results section was well structured but I found the section on prevention studies and the differential effects according to illness severity hard to follow.

RESPONSE: This paragraph has been rewritten to articulate more clearly the relative risk (RR) and absolute risk reductions (ARR) for mild and moderate severity RTI.

It now states: “The largest reductions in relative risk were for moderately severe symptoms consistent with a flu-like illness (e.g. elevated temperature). There was an 87% lower risk of developing moderately severe symptoms (IRR 0.13, 95% CI 0.04 to 0.38, NTT=100) compared to a 28% lower risk of developing milder symptoms (e.g. common cold) (IRR 0.72, 95% CI 0.61 to 0.85, NNT=25).[73-75] However, due to more people contracting mild RTIs, the absolute risk reduction/risk difference was higher. Five mild RTIs were prevented per 100 person-months (95% CI 2 to 7) compared to one moderate RTI per 100 person-months (95% CI 1 to 2) (Appendix 4).[73-75]”

**2.** The findings regarding symptom severity are inconsistent with the effect on 3 day severity reaching clinical significance where-as the effect on average daily symptom scores was nonsignificant.

RESPONSE: We have added this non-significant finding to the abstract and also to first section in the discussion where we also state "The different findings may reflect reporting bias as results could not be extracted for 13 [55 56 63-71 76] of the 20 RCTs [55-71 76] that evaluated symptom severity, and of the 7 RCTs [55-61] in the two meta-analyses, only one RCT [57] overlapped."

**3.** The clinical significance of the 3 day score was only agreed post hoc and this might be considered in the limitations.

RESPONSE: In Box 1 and Discussion/Strengths and Weakness of the Review, the risk of reporting bias from any post-hoc changes has been added. In the Methods, additional information about how the clinical significance, that is the minimally important difference (MID) was determined is briefly noted and now signposts the reader to Appendix 4 where the details for the calculations were reported. The Limitations in the Discussion now includes an additional paragraph about protocol changes and post hoc decisions.

The last bullet point in Box 1 now states: "Protocol changes and post hoc decisions are declared; however, this increases the risk of selective reporting bias."'

The Methods/Statistical methods and evidence synthesis, now states: "For symptom severity on day3 for mild RTIs, the MID for mean difference was set at 1 point on a standardized scale that was the half-way mark between two proposed MIDs (Appendix 4).[47 48]"

The Discussion/Limitations now states: "Finally, whilst all protocol changes were declared, this increases the risk of selective reporting bias. Initiating the rapid review in the early stages of the Covid-19 pandemic necessitated rapid protocol development and registration. To help mitigate bias we sought blinded advice from our content experts and consumer/patient advocates and post hoc decisions were conservative with the rationale reported."

4. Abstract The abstract makes no mention that this paper is presenting the secondary outcomes for a review focussed on the role of zinc in ameliorating Covid-19 infection. The rapid review has been adapted from that originally described in the Prospero registration including changing limits to some searches. This should be made clear at some point in the abstract. The same explanation should be included in the limitations section in the discussion.

RESPONSE: In the context of this pandemic, due to very serious concerns with the indirectness of the available evidence (i.e. no SARS-CoV-2 RCTs) we think it is important not to overstate the relevance of our findings. It is for this reason that we removed COVID-19 from the title and did not GRADE the certainty of the evidence in the context of SARS-CoV-2 prevention or treatment. However, in the Abstract we now clarify that the extended searches were only for SARS-CoV-2 RCTs and that this was the primary population of interest. The manuscript now contains more information about these post hoc decisions and limitations.

The Abstract now states in the Methods: "Seventeen English and Chinese databases were searched in April/May-2020 for randomized control trials (RCTs) and to August-2020 for SARS-CoV-2 RCTs, the primary population of interest." and in the Results: "None were specific to SARS-CoV-2."

In the manuscript, the Methods/Protocol now states: “Due to very serious concerns with the indirectness of the available evidence and the importance of not overstating its relevance to the pandemic, the post-hoc decision was made to remove COVID-19 from the title and not GRADE the certainty of the evidence in the context of SARS-CoV-2 prevention or treatment.”

The Methods/Protocol also now states: “Further details about protocol and post hoc changes are reported below and in Appendix 4.” Due to word count limits, Appendix 4 now contains extensive details.

The Methods/Search strategy now states: “This was supplemented by bibliography searches of included articles, and due to no eligible RCTs being identified in the first search additional post hoc covid-19 focused searches were conducted up to 19 August 2020 that included the addition of medRxiv and bioRxiv pre-print databases.”

The Results/Study Characteristics now states: “None were infected with the primary pathogen of interest SARS-CoV-2.” and further down when reporting which of the critical (primary) and important (secondary) outcomes were reported by the included RCTs, its now states: “All but two RCTs reported at least one result that was used in a meta-analysis of a critical or important outcome.[54 76] None of the RCTs reported mortality or other clinical outcomes relevant to severe or critical illness from acute viral RTIs, or quality of life outcomes.”

In the Discussion, the first paragraph now reiterates this negative finding by stating: “Further, despite additional searches through to August 2020, none of the RCTs were for the primary population of interest that was SARS-CoV-2 infection as the results from the registered clinical trials were all pending.”

It is also acknowledged as a limitation in the section - Strengths and Weakness of the Review that now ends with an additional paragraph about post-protocol changes. The paragraph opens by stating: “Finally, whilst all protocol changes were declared, this increases the risk of selective reporting bias.” And ends with: “It also ensured that the very serious indirectness of the available evidence was not overstated in the context of SARS-CoV-2 prevention or treatment.”

**5.** The third bullet point in strengths and limitations is missing some text

RESPONSE: At the request of the editors, the bullet points have been extensively edited. Please see the above response to the editors.

**6.** Discussion The findings are appropriately summarised. Again I find this sentence (line 8) hard to follow which relates to the differential findings for illness severity This risk was substantially lower for preventing more severe symptoms such as fever.

RESPONSE: The sentence (line 8) has been changed to: “New evidence about zinc prophylaxis found that compared to placebo there was a reduced risk of developing symptoms consistent with a community acquired viral RTI. The prophylactic effects were greatest for reducing the relative risk of developing more severe symptoms, such as fever and flu-like illnesses.”

**7.** The implications for clinicians may be overstated given the uncertainty over effects on acute illness severity/duration. These uncertainties are explored in the subsequent paragraph.

RESPONSE: The first section of the Discussion has been substantially revised so that the many uncertainties in the evidence are highlighted from the outset.

**8.** Figure 4 was hard to read

RESPONSE: The figure has been revised. Rather than presenting the meta-analyses of hazard ratios and mean days difference next to each other horizontally, they are now presented separately in a sequential vertical order.

Reviewer: 2
Dr. Peter Wilson, University College London Hospitals NHS Foundation Trust Comments to the Author:

This is a systematic review and meta-analysis in an area of potential interest during the pandemic. There was an increase in mild adverse effects but there was a reduction in viral respiratory infection or the symptoms. The review follows Cochrane guidance and PRISMA and involved patient representatives. The databases searched were wide ranging.

**9.** Data review was not by two reviewers in all cases, but calibration was used between reviewers.

RESPONSE: Since the submission over 6-months ago, all reported results have been meticulously checked for calibration, data extraction and copy errors.

We found a unit-of-analysis error in the meta-analyses of the two 4-arm RCTs that compared zinc to an active control lozenge. This resulted in small, insignificant conflations from the unaddressed correlations. This error has been corrected. We report sensitivity analyses in Appendix 4, as more than one analytical approach is possible.

We had missed that two of the included RCTs reporting symptom severity, also reported the postexposure prophylactic (PrEP) effects of zinc lozenges following human rhinovirus (HRV) inoculations. The data were extracted and added to Appendix 3/Characteristics of Studies table and the metaanalysis of zinc for prevention of a clinical cold following HRV inoculations. This includes a sub-group analysis for PrEP and post-exposure prophylactic (PEP) studies. Again, the overall findings/conclusions are unchanged.

We decided to apply recommended systematic review methods where two reviewers independently appraise the RoB. The adjustments to the RoB assessments did not change our GRADEcertainty/quality assessments nor our conclusions. The methods have been edited and now state: “Single reviewers appraised the risk of bias (RoB) of study outcomes with the Cochrane RoB 2.0 tool[37] that was verified by a second review. However, discrepancies in calibration led to the post hoc decision to apply recommended systematic review methods where two reviewers independently appraise the RoB.”

**10.** GRADE was used in assessing evidence but a quality index was not.

RESPONSE: Respectfully, we do not understand why the reviewer thinks a quality index was not used. GRADE is a quality index. According to clarifications made by the GRADE Working Group in 2017, please “Note that “quality of evidence” refers to the same concept as “certainty of evidence”,” and “In the context of a systematic review, the ratings of the quality of evidence reflect the extent of our confidence that the estimates of the effect are correct.” (Box 1: https://doi.org/10.1016/j.jclinepi.2017.05.006)

To help clarify, throughout the manuscript we have inserted the term “/quality” alongside “certainty”.

**11.** The results seem sound and conclusive in respective of a preventative effect and a reduction in symptoms of infection. The review makes a contribution to knowledge beyond earlier reviews in the scope of papers covered. Anosmia as an adverse effect is clearly of concern during the Covid-19 pandemic as diagnosis will be compromised and should be discussed.

RESPONSE: The following sentences have been added in the Discussion: Implications for clinicians and consumers. “Notwithstanding, anosmia is an early SARS-CoV-2 symptom, so any use of topical nasal zinc during the pandemic should be carefully considered and monitored.”

**12.** The use of a single albeit calibrated reviewer does carry a small risk of error.

RESPONSE: We agree, which is why this risk is now also acknowledged as a weakness in Box 1.

**13.** The conclusions appear to be justified and the use of zinc may be a useful preventive measure in some patients. However, it would be helpful to have some practical recommendations for future practice, particularly as this may be self-selected and applied by the public without medical intervention.

RESPONSE: Thank you for this suggestion. Recommendations for clinical practice should be informed by more than just the certainty/quality of the evidence. Other important considerations include patient preferences, costs (financial and opportunity), benefits vs risks, availability/access to interventions etc. All of which are beyond the scope of this review.

As such we have added the following to the conclusion: “Uncertainty remains about the comparative efficacy, effectiveness and acceptability of different zinc formulations and doses, and their mechanisms of action. This review did not evaluate patient preferences, financial and opportunity costs, and availability of different zinc interventions that should also be considered prior to recommending zinc to prevent or treat viral RTIs.”

**14.** The protocol provided reflects the original plan to examine coronavirus rather than the revised version submitted which includes all viral respiratory infections. This should be clarified. BMJ Open published all the methods in 2020. Full documentation has been provided for search strategies, excluded and included references.

RESPONSE: The published changes to the PROSPERO protocol led to RCTs evaluating zinc for prevention and/or treatment of infections following HRV inoculation being included. All the other RCTs that were included met our original inclusion criteria registered on PROSPERO, as they were all non-specific community acquired RTIs that can be caused by coronavirus infections.

Due to word count limits Appendix 4 now provides further clarification about the changes made to the inclusion and exclusion criteria. It states: “The protocol was first registered on PROSPERO CRD42020182044.[1] Following feedback from our content experts who at that stage were blinded to the search results, amendments were made pre-data extraction and a revised protocol was submitted for publication (Supplementary file: protocols).[2] This included expanding the inclusion criteria from only including RTIs that can be caused by a coronavirus to RTIs caused by any virus (e.g. rhinovirus inoculation), tightening the exclusion criteria to exclude respiratory illnesses not predominantly caused by viral infections (e.g. pneumonia in adults) unless a viral infection is confirmed, and finalising the measures of effect to be extracted for the a priori outcomes. For pragmatic reasons, the post hoc decision was made to only extract data for adult populations.”

In the last paragraph that was added to the Discussion/ Strengths and weakness of the review, it includes: “Notably, whilst the published ammendments[33] to the protocol inclusion/exclusion criteria led to RCTs that inoculated participants with HRV also being included, the additional findings did not favour zinc. Post hoc decisions also ensured that the very serious indirectness of the available evidence was not overstated in the context of SARS-CoV-2 prevention or treatment.”

Reviewer: 3
Dr. Surjit Singh, AIIMS Jodphur Comments to the Author:
A Very well written study. A timely done study for facilitating the use of Zinc for COVID-19.

**15.** Risk of bias

RESPONSE: Thank you for your detailed feedback about RoB. Since submitting this manuscript, we decided to apply recommended systematic review methods where two reviewers independently appraise the RoB and have adjusted our RoB assessments accordingly. We anticipate these adjustments will address many of your concerns below. In Appendix 3, Table 3 replaces Figure 2. In Appendix 4, the sensitivity analyses for RoB were recalculated in response to any changes to the RoB assessments. The changes to the individual RoB assessments did not change the GRADEcertainty/quality assessments or our conclusions.

**16.** Risk of bias. It is usually understood that risk of bias for adverse effects should be low risk of bias even it is subjective. As adverse events are reported by patients, less likelihood of bias.

RESPONSE: Thank you for this guidance. Our review of our RoB assessments mostly led to revisions to the AE RoB assessments.

For Domain 4. Measurement of the outcome, blinding of the outcome assessment was only one factor we considered when assessing the RoB. As per published guidance, those rated as a high RoB were due issues other than blinding, for example, recall bias or passive reporting. Question 4.3 asks if the outcome assessors were aware of the intervention received by study participants. We answered NO for AEs if the assessor(s) were blinded, and adequacy of blinding was assessed as preserved. Based on this, only two AE assessments were rated as having Some Concerns rather than a low RoB for Domain 4. However, in both instances the placebo lozenge was matched for appearance and excipient ingredients. Based on your feedback, we therefore consider it is also reasonable to answer NO for question 4.3 and have now rated them both as Low RoB for Domain 4.

**17.** I was not able to understand the following differences in ROB in adverse events: Eby 1984 – Why it is some concerns with randomization despite low ROB in duration and severity. According to me it is not possible.

RESPONSE: We agree. Following revision of RoB 2.0, the adverse event outcome for Eby 1984 was re-appraised as a High RoB for Domain 1. Randomisation process. It is now the same as the RoB for the study’s duration and severity outcomes.

**18.** There are some differences in ROB assessed with regard to adverse events and other efficacy outcomes. This is quiet unlikely to happen as it is rare to have missed data for AE, issues with randomization or blinding if it is not there for efficacy outcomes.

RESPONSE: Differences in the RoB assessments for outcomes from the same study is a feature of RoB 2.0. As per our earlier response (no. 17) we agree that issues with randomization (Domain 1)

should be the same irrespective of the outcome. Similarly, we agree that issues with blinding of participants and personnel are mostly the same irrespective of the outcome. However, this is only one factor that is considered when assessing Domain 2 - Deviations from intended interventions, and Domain 4 - Measurement of the outcome. As per our earlier response (no. 16) we note that the RoB from blinding of outcome assessments may differ when assessing Domain 4. Domain 3 - Missing outcome data can also differ, for example, we identified instances where data from participants who withdrew from the study were excluded from the analysis of efficacy outcomes yet included in the AEs analysis. Further details of our RoB assessments is now provided in Appendix 3. Table 3: RoB for each study outcome.

**19.** Results. Figure 2: Kindly check the data of Wei 2009 and Zhang 2009 as this data only has shown significant result because of which we have evidence of efficacy. I was unable to retrieve the articles. If you have kindly upload them next time so that I can check the data of Community acquired mild to moderate RTI.

RESPONSE: The pdf files are uploaded. A native Chinese speaking researcher extracted the data and appraised the RoB. A second reviewer used Google translate to verify and this is now noted in the Methods. Data extraction and the calculations of incidence rate per-person months have been added to Appendix 4.

We identified a copy error for number of events in the control group for Zhang 2009. This led to a slight underestimation of reduced relative risk that has been corrected from 0.69 [95% CI 0.60, 0.80] to 0.68 [95% CI 0.58, 0.80].

**20.** I do not completely agree with the GRADE of moderate quality with regard to this outcome. No publication bias assumed does not apply to this outcome as only four studies were reporting the data. Hence there is publication bias which will downgrade the evidence to low rather than moderate. Kindly discuss with colleagues and some other experts in systematic reviews.

RESPONSE: According to GRADE guidance (Guyatt et al. 2011), “consider rating down for likelihood of publication bias when the evidence consists of a number of small studies” (p 1279). This guidance is referring to the number of studies with small sample sizes rather than a small number of studies in the meta-analysis. GRADE acknowledges the difficulties in assessing the likelihood of publication bias and as such recommends only rating down by one level when publication bias is ‘‘strongly suspected" (p 1281). None of the outcomes we report meet the GRADE criteria and as such do not warrant rating down for publication bias.

Guyatt GH, Oxman AD, Montori V, et al. GRADE guidelines: 5. Rating the quality of evidence-publication bias. J Clin Epidemiol 2011;64(12):1277-82.

**21.** No publication as assessed by Eggers regression test is of outcomes which were reported by majority of studies.

RESPONSE: Due to word count limits, details of the assessments of publication bias results, both visual inspection of funnel plots and statistical tests, are reported in Appendix 4.

**22.** Serious Adverse events –It should be mentioned that studies did not report the SAE if they did not have mentioned it. If studies have mentioned that there are no serious AE only then the conclusion drawn by authors is correct. Kindly check it.

RESPONSE: Thank you we have checked that Serious Adverse events were included in the reporting of any adverse event.

To improve clarity, we have changed the terminology from adverse effects to events and edited the Results in the Abstract and Manuscript.

The Abstract/Result now states: “No serious AEs were reported in the 24 RCTs that reported AEs.”

The Manuscript/Results now states: “No serious adverse events were reported were reported in the four RCTs that reported AEs when zinc was used for preventing community acquired RTIs.” and: “No serious AEs were reported in the 19 RCTs that reported AEs for treating RTIs.”

No changes were made to the reporting of the two safety/tolerance studies.

**23.** In addition if no SAE were reported then ROB should be high as all the studies data were included. Therefore, overall Low evidence quality for SAE.

RESPONSE: All RCTs that reported AEs were appraised for selective reporting bias of adverse events, including SAEs, in Domain 5 (RoB 2.0 tool). There were two typos with the reporting of the SAEs in the Summary of Findings table (Appendix 5). For RoB Not Serious has been corrected to Serious and the overall GRADE assessment should have been Low Certainty/Quality evidence due to Serious RoB (rated down 1 level) and Serious Imprecision (rated down 1 level).

**24.** Appendix: Meta-analysis results (4.1) on page 6. Mean duration of symptoms are positive days while 95% CI is negative or decrease in days. Kindly check.

RESPONSE: Thank you, we have checked and corrected typos.

**25.** Funnel plots Figure – All three figures: Visual inspection is suggestive of asymmetry. However Egger’s regression test or Harbord score showed that there is no publication bias. This should be your conclusion.

RESPONSE: Visual inspection of funnel plots are necessarily subjective. To aid this assessment we in Appendix 4 we have replaced the funnel plots with contour-enhanced funnel plots. Accordingly, we agree that all three figures are suggestive of asymmetry. For the interpretation of the funnel plots, we refer to Sterne et al. 2011. When taken overall, we did not consider that the inspections and statistical tests presented convincing evidence of publication bias (i.e. small study bias). Published GRADE guidance recommends only rating down for publication bias when it "strongly suspected".

In Appendix 4, the RevMan generated funnel plots have been replaced, and the interpretive text beneath each figure is edited and now states:

For Hazard Ratios – “Visual inspection of the funnel plot is suggestive of asymmetry. However, the outlying study with the largest effect size, also had the largest sample size (n=213).[31] This is inconsistent with publication bias from favourable small studies. Heterogeneity can also cause funnel plot asymmetry.[16] Removal of this study outlier substantially reduced this asymmetry and statistical heterogeneity reduced from $I^2$=82% to 60% (Table 4.1.11. symptomatic risk). Overall, small study bias is not strongly suspected. ”

For Mean Difference – “Visual inspection of the funnel plot shows asymmetry that is suggestive of small study bias. However, there was considerable heterogeneity ($I^2$=97%) that can confound the

interpretation of funnel plot asymmetry[16] and Egger's regression was not significant (p = 0.54). Overall, small study bias is not strongly suspected."
For Adverse Events – "Visual inspection of the funnel plot showed some asymmetry. However, the asymmetry is in favour of placebo controls. This is the opposite of what is expected when there is publication bias from favourable small studies. The Harbord score was not significant (p = 0.073). Overall, small study bias is not strongly suspected."
In the Results/ Certainty and quality of the evidence, we now state: "Visual inspections of the three funnel plots with 10 or more RCTs showed some asymmetry. However, Egger's regression was not significant (p = 0.54) for mean days duration, the Harbord score was not significant (p = 0.073) for risk of non-serious adverse effects, and heterogeneity[16] rather than small study bias was suspected as the main reason for the asymmetry in the hazard ratio meta-analysis publication bias (Appendix 4). For the other outcomes, less than 10 RCTs were included in the meta-analyses. As such, publication bias was not strongly suspected for any of the outcomes."
In the Discussion under limitations, we now state that "However, there were limitations to our GRADE assessments. For instance, while publication bias was not strongly suspected, visual inspection of funnel plots are necessarily subjective and a statistical test for hazard ratios was not performed.[53]"
[16] Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ. 2011;343:d4002.

**26.** Page 108 – 6.5 – Days taken for improvement Data of Mossad 2003. Medians are taken as means as per Cochrane. Cochrane Handbook said that SD should be calculated as IOR/1.35 which comes out to be 2.2 in zinc and 2.59 in control group instead of 0.75 and 0.88 as mentioned by you. I have calculated it from article of Mossad et al 2003. Kindly enter the correct data for synthesis of plots.

RESPONSE: The sources for our data extraction for mean days duration is reported in Appendix 4. The Mossad 2003 data was extracted from a systematic review by D'Cruze et al. 2009.[36] The reviewers contacted the authors and were advised that the mean days duration (SD) for the zinc group was 4.3 days (SD 0.75) and placebo group was 6 days (SD 0.88).

D'Cruze H, Arroll B, Kenealy T. Is intranasal zinc effective and safe for the common cold? A systematic review and meta-analysis. Journal of primary health care. 2009;1(2):134-139.

**27.** GRADE. Overall moderate quality of evidence for acute viral RTI and SAE should be checked and discussed as both seemed to be of low quality.

RESPONSE: As per our response above, we agree the GRADE-certainty/quality of the evidence for SAE is low due to serious RoB and serious imprecision, this was a typo and moderate has been changed to low in the Summary of Findings table in Appendix 5. We did not report the certainty/quality of this evidence in the manuscript, so this has now been added whenever SAE are reported.

However, we stand by our assessment of moderate certainty/quality evidence for the prevention of symptoms consistent with an acute viral RTI. There were no serious concerns with inconsistency, indirectness, imprecision, or publication bias. For publication bias we defer to our earlier response to no. 20. Three of the four RCTs had some concerns with RoB and the other a high RoB. The effect estimate was stable when the RCT with a high RoB was removed: IRR 0.68 [95% CI 0.56 to 0.81] p < 0.001 compared to IRR 0.68 [95% CI 0.58 to 0.80] p < 0.001. We therefore rated down the GRADEcertainty by one level rather than two levels for risk of bias.

For zinc compared to placebo, we agree that the GRADE-certainty/quality of the evidence for day-3 symptom severity, average daily symptom severity, and the risk of remaining symptomatic is low.

However, we have reconsidered our GRADE-certainty/quality assessment for mean days duration from zinc compared to placebo and recommend changing the rating from low to very low certainty/quality due very serious concerns with inconsistency. There is considerable statistical heterogeneity that unlike the meta-analysis of the hazard ratios, it cannot be adequately explained by clinical or methodological diversity. There are also wide variations in the point estimates and minimal overlapping of 95% CI. As such we suggest rating down by two levels, rather than one level. Combined with rating down one level due to serious concerns about RoB, the GRADE assessment would be very low certainty/quality.

Additional details of the GRADE-certainty/quality assessments have been added to the footnote of the Summary of Findings table in Appendix 5.

Reviewer: 4
Dr. Chun-Yu Liang, National Defense Medical Center Comments to the Author:
Overall, this is a clear, concise, meaningful, and well-written manuscript. There are few comments as followed.

**28.** According to PRISMA 2020 guideline, it is appropriate to measured consistency using Cochran Q text and $I^2$ in each subgroup analysis.

RESPONSE: Further details about the interpretation of Cochran Q text and $I^2$ statistic for the subgroup and sensitivity analyses are now reported in Appendix 4.

We have also updated our checklist to the PRIMSA 2020.

**29.** p7, it is suggested to add the statistical methods using in meta-regression for the outcome of RTIs.

RESPONSE: These were reported in Appendix 4. They have now also been added to the manuscript that states: “The Mantel-Haenszel method was used to calculate the pooled RR, generic inverse variance method was used for MD, SMD and IRR, and O-E variance method was used for HR. Irrespective of statistical heterogeneity, due to considerable clinical and methodological diversity/heterogeneity, random effects models were used.”

**30.** Appendix 4, the p-value= 0.000 should change to p-value < .001 in tables.

RESPONSE: These have all been changed in accordance with BMJ Open style p<0.001

**31.** p94, please explains more about " When no RTIs were reported in one study arm, 0.5 was recorded to facilitate analysis." Why used the number of 0.5 and how to avoid bias?

RESPONSE: How best to handle zeros in meta-analysis remains a contentious issue. The approach we used is recommended in the Cochrane Handbook 10.4.4.1. Nevertheless, we recognise it may bias the study estimates and/or over-estimate variances of study estimates (consequently downweighting inappropriately their contribution to the meta-analysis). The risk of bias is greatest when the sizes of the study arms are unequal, it is a rare event, there are no events in more than one arm, there are a lot of zeros in the meta-analysis, and there is high heterogeneity.

We had two meta-analyses where a zero count was replaced with 0.5 in one of the study arms. In both instances there was only one arm with zero events, the zinc and placebo arms were an equivalent size, it was not a rare event (the rates in the placebo arms were both 0.1) and there was

low ($I^2$=0%) or moderate ($I^2$=62%) heterogeneity that was not influenced by the inclusion/exclusion of the study with a zero count in one arm.

Replacing the zero count with a smaller number dramatically increases the SE and down-weights its contribution to the meta-analysis and vice versa. For instance, in the meta-analysis of zinc for preventing moderate RTIs, when zero is replaced with 0.5 the IRR 0.13 (0.04, 0.38) and the study contributes 13.8% weight to the meta-analysis, with 0.1 the IRR reduces to 0.11 (0.04, 0.37) with a 3.5% weight, with 1.0 the IRR increases to 0.15 (0.05, 0.43) with a 21.9% weight. We therefore consider the recommended Cochrane approach to is the most appropriate for our data.

In Appendix 4, to further clarify why zero events were replaced with 0.5, we have changed the sentence to the following: "In one RCT there were no moderate RTI events in the zinc arm.[4] In another no adverse events were reported in the zinc arm.[5] In both instances, it was not a rare event, the study arm sizes were balanced, there was only one zero cell in the meta-analysis, and metaanalysis heterogeneity was acceptable. Therefore, as per Cochrane guidance, 0.5 was recorded to facilitate analysis.[7] "

32. p 100 figure 1, please show the result of Egger's regression for the test of bias.

RESPONSE: We thank the reviewer for their suggestion to conduct a statistical test of publication bias on the Likelihood of Recovery outcome. This outcome is a hazard ratio and accepted statistical approaches to explore publication bias for survival data are still in the development phase (Debray 2017). Considering the lack of consensus in the evidence-synthesis methodological field on this issue, we opted not to conduct statistical tests on HR outcomes and have therefore relied on the visual inspection of the funnel plot only.

We now note this with the reference in the Methods/Statistical methods and evidence synthesis that now states: However, due to ongoing methodological uncertainties, no statistical test was used for hazard ratios.[53]"

This limitation is now noted in the Discussion/Strengths and weakness of the review that now states: "However, the assessments of publication bias were a potential limitation. While publication bias was not strongly suspected, visual inspection of funnel plots are necessarily subjective[52] and a statistical test for hazard ratios was not performed."

[52] Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ. 2011;343:d4002.

[53] Debray TPA, Moons KGM, Riley RD. Detecting small-study effects and funnel plot asymmetry in meta-analysis of survival data: A comparison of new and existing tests. Research synthesis methods. 2018;9(1):41-50.

## VERSION 2 – REVIEW

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| **REVIEWER** | Wilson, Peter<br>University College London Hospitals NHS Foundation Trust, Microbiology |
| **REVIEW RETURNED** | 15-Aug-2021 |

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| **GENERAL COMMENTS** | Thank you for making the revisions and clarifying where appropriate. I think the issues are resolved. |

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| **REVIEWER** | Singh, Surjit<br>AIIMS Jodphur, Pharmacology |
| **REVIEW RETURNED** | 18-Aug-2021 |

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| **GENERAL COMMENTS** | Dear Authors<br><br>A very well conducted review.<br><br>With regard to adverse events quality (GRADE), it can be mentioned that absence of serious events in studies have resulted in small effect, which has resulted in low quality. However, the moderate quality of evidence for serious adverse event cannot be ruled out.<br><br>Figure 2 in first version is missing in current manuscript. You can make the ROB-2 image from ROB visualization tool site. https://www.riskofbias.info/welcome/robvis-visualization-tool. Please add the ROB image, although ROB images are added along with forest plots. |

**VERSION 2 – AUTHOR RESPONSE**

Reviewer: 3

3. With regard to adverse events quality (GRADE), it can be mentioned that absence of serious events in studies have resulted in small effect, which has resulted in low quality. However, the moderate quality of evidence for serious adverse event can not be ruled out.

RESPONSE: We acknowledge that factors informing quality/certainty decisions necessitate a degree of judgement and this may vary between different systematic reviewers. We strived to apply a consistent approach by establishing rubrics to guide calibration for our RoB and GRADE assessments. For the sake of consistency with our other GRADE-assessments, we are inclined to stand by our assessment of low certainty/quality evidence and instead have acknowledged the reviewer's feedback that "moderate quality of evidence for serious adverse event can not be ruled out".

For the GRADE assessment, we rated down 1 level for imprecision as the optimum information size was not met and important risks of SAEs could not be excluded. We set the criteria for no serious concerns for risk of bias (RoB) at 75% of studies having a low RoB. Serious adverse events (SAEs) were rated down 1 level as only 7 of the 25 RCTs that evaluated SAEs had a low RoB for adverse events. As per published RoB 2.0 guidance, those rated as a high RoB were due bias from the randomization process (9 RCTs), deviations from the intended intervention (6 RCTs), missing outcome data (11 RCTs), measurement bias (5 RCTs), and selective reporting (11 RCTs). Combined, according to the GRADE guidelines, our decision to rate down by 2 levels meant our overall assessment was low rather than moderate certainty/quality evidence. However, we appreciate

that the RoB 2.0, like many RoB appraisal tools, was developed for appraising efficacy outcomes and there are calls to develop RoB tools specific for assessing the RoB of adverse drug events (DOI: 10.1016/j.jclinepi.2017.04.023). As such, we appreciated that other reviewers might not to downrate for RoB and then assess the GRADE-certainty of the evidence as moderate.

In the discussion section, under Strengths and Limitations, the following has been added:

“RoB appraisal at the outcome level rather than the study level helped optimise GRADE-certainty assessments that were both conducted following calibration exercises. Notwithstanding, there is always a degree of judgement that may vary between reviews. For instance, when appraising the available RCT evidence for risk of serious adverse events, we rated down one level for RoB and another for imprecision. However, it might also be reasonable to judge the RoB as not serious and the overall GRADE assessment as moderate, rather than low certainty/quality evidence.”

We had already noted in the Discussion that post-market surveillance has identified permanent anosmia (loss of smell) from topical nasal zinc and highlighted our concerns with the included RCT evidence that assessed the risk of copper deficiency from oral zinc use. This has been extended. In the Discussion section: Implications for clinicians and consumers, the third paragraph has been extensively edited and now includes a clear statement that whilst the risk of SAEs is low, it cannot be ruled out. It now states:

"… Zinc was found to increase the risk of non-serious adverse events. No serious adverse events were reported, suggesting the risk is low. However, it cannot be ruled out as RCTs, especially those with small samples, are not well placed to identify rare events. If the rule of three is applied to determine maximum risk,[100] then the upper 95% CI for a serious adverse event from prophylactic zinc would be 1.7/1,000 person-months and for therapeutic zinc, 2.9/1,000 participants. Indeed, post marketing surveillance has identified cases of long-lasting anosmia associated with a zinc gluconate nasal gel.[101,102]...”

Reference 100 is new:

Eypasch, E., Lefering, R., Kum, C. K., & Troidl, H. (1995). Probability Of Adverse Events That Have Not Yet Occurred: A Statistical Reminder. BMJ: British Medical Journal, 311(7005), 619-620

In the Conclusions, the sentence about AEs now states:

“Whilst there was an increased risk of non-serious adverse events that may limit tolerability for some, the risk of serious adverse events was low.”

In response to the editor’s feedback about the conclusions, we have removed all statements about the certainty/quality of the available evidence.

4. Figure 2 in first version is missing. You can make the ROB-2 image from ROB visualization tool site. https://www.riskofbias.info/welcome/robvis-visualization-tool.

RESPONSE: respectfully, due to the limit of 5 figures and tables, an overall RoB figure was never included in the manuscript and has only ever been presented in the supplementary file (Appendix 3). In both the original submission and revision 1, Figure 2 has always presented the Forest plots for prophylactic zinc. Like Figures 3, 4, and 5, next to the Forest plots, the individual RoB assessment for each RCT outcome is presented.

With the editor's permission, we would be pleased to move the RoB-2 figure from the appendix to the manuscript.

## VERSION 3 – REVIEW

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| **REVIEWER** | Singh, Surjit<br>AIIMS Jodphur, Pharmacology |
| **REVIEW RETURNED** | 06-Sep-2021 |

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| **GENERAL COMMENTS** | Very Good review. You should write a paper on how to synthesize plots (Forest and funnel) plots in R. or Kindly share in the supplementary file so that other people can get benefitted from the article.<br><br>Best Wishews |

## VERSION 3 – AUTHOR RESPONSE

Reviewer: 3 - You should write a paper on how to synthesize plots (Forest and funnel) plots in R. or Kindly share in the supplementary file so that other people can get benefitted from the article.

RESPONSE: To clarify, all Forest plots were generated in RevMan.

The R codes used to synthesize the contour enhanced funnel plots have been added to Supplementary File: Appendix 4. section 6.7

A paper describing how to perform a meta-analysis with R, including how to generate Forest and funnel plots has been added to the reference list. It is now cited in the methods section of the manuscript (reference no. 42) and also in Appendix 4 (reference no. 5).

Balduzzi S, Rücker G, Schwarzer G: How to perform a meta-analysis with R: a practical tutorial. Evid Based Ment Health 2019, 22(4):153-160.