PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Occupational risk for Legionella infection among dental healthcare workers: Meta-analysis in occupational epidemiology
AUTHORS	Petti, Stefano; Vitali, Matteo

VERSION 1 - REVIEW

REVIEWER	Norman Noah London School of Hygiene and Tropical Medicine London UK I know one of these authors, having met him many years ago.
REVIEW RETURNED	14-Dec-2016

GENERAL COMMENTS	This is a well-conducted meta-analysis on a difficult subject. The first problem is the limitation of such a small number of studies available that they could work on = 7 or 8 depending on how you count them. Second, there is a very wide variation in findings in these 8 studies - but perhaps that is what a meta-analysis is about. As the authors point out, there is a significant publication bias as well. However two studies used, especially the one from Austria, and also the 'half' one from Bari in Italy, provide results way outside the others. Thus there is not only large variation between countries, but even within countries - e.g the Borella paper from Turin and Bari. Furthermore, LD is influenced by climate, season, and other environmental factors so that nothing is in the end, static. I can't help feeling in the end that one great message of this paper is precisely that: a meta-analysis may be helpful but it is really the responsibility of each country, even every region within a country, to do their own assessments. My only other major point is that the authors should maybe focus more on the difference before and after the CDC guidelines on Infection Control in LD. These were published in 1993, and that authors have very sensibly chosen 1998 as their 'cut-off' point. There is a nice message here that whatever risk there was to dental HCWs before the guidelines, there is some evidence that they have made a difference. They could almost make this as their major objective. It suggests that the risk can be modified substantially. Some minor comments: - an overuse of 'among' rather than 'in'. - I am not sure what they mean in the Abstract/Conclusions by including 'the practical contribution of manufacturers' as I do not think this is discussed in the text - the English, with a few Americanisms thrown in, and some

	punctuation problems will probably be dealt with by your copy-editors - in the first para of the Introduction, they should give dates as years; this para includes 'Just few years later.....'; 'in the same period.....'; 'a decade later.....'. I was lost long before that last decade! - the firs para of Methods would be better in the Discussion. - towards the end of results, on p 9, they say the 'two subgroup analyses were planned as follows' and then follow this with an incomprehensible paragraph. State exactly what the two groups were after ' as follows'. What these two groups were could be clarified. All in all, however, the authors make a good case for dental staff not being at great risk of acquiring LD. However possibly vigilance is necessary, as is following good hygiene practices as per existing guidelines from reputable sources.

REVIEWER	Haluk Erdogan Baskent University, Department of Infectious Disease and Clinical Microbiology, Turkey.
REVIEW RETURNED	03-Jan-2017

GENERAL COMMENTS	"Literature search was performed by one Author (SP)" may have potential risk of bias. Authors should also focus on the main findings of the study in the discussion section.

REVIEWER	Chris Robertson Strathclyde University, Glasgow Scotland
REVIEW RETURNED	09-Feb-2017

GENERAL COMMENTS	This is a very detailed meta analysis of the risk of carrying legionella antibodies among dental health workers in relation to controls who are occupational but not likely to be exposed to legionella. I think that this study appears to have been carried out in a careful fashion. The statistical aspects of the Meta Analysis are standard and are carried out in a very clear and detailed fashion. I think that this is a complex analysis and by and large I think that this work has been well written up. It was a little bit difficult for me to understand exactly what had been done in the selection of the studies and this needs to be clarified. In each study selected the prevalence in dental workers is compared to a control group and while there is a list of occupations excluded from the control group, exactly who are the controls in each study were not presented. I think that this is essential and should be listed. Also it was not completely clear to me if the study was just about estimating prevalence, in which case you could have single arm studies, or about comparing prevalence in DHCWs to a control. I

	concluded that it was the latter but initially was not sure. More detailed comments Abstract Results The pooled PR was statistically non-significant at 95% level (1.7; 95CI, 0.8-3.2), I do not understand this statement and this that you would be better to clarify that this is the PR comparing exposed DHCWs with unexposed. Also I am not sure what the unexposed group is. Later on you state that all DHCWs are exposed Page 3 line 11. If the risk is negligible why would you want to ascribe it to anything. I do not really see why you would then attempt to ascribe it to any factor. P6 line 51. I think you should clarify when case control studies were part of the search as the main aim seems to be to ascertain prevalence of legionella among dental workers. You will get risk from the cohort and cross sectional studies. It has just occurred to me that you might be looking to compare prevalence in different groups of workers and this has not really been clear from the introduction. I think it would be better to make this clear from the start – you are looking for studies where you can compare the prevalence of legionella in DHCWs with another group – At this stage I am not too sure what the other group is – unexposed DHCWs or another unexposed group. Page 6 Inclusion/exclusion criteria. I am afraid I found these quite difficult to comprehend and think that they need to be expressed in a clearer fashion. 1 is fine but 2-5 are not really inclusion/exclusion criteria for studies. I think that the rest mean that within each study you have to have sufficient information to be able to identify a group of exposed DHCWs and a controls group of workers who are not DHCWs and who are not likely to be exposed to legionella through their jobs P 6 l 19-35 please state explicitly why each occupational group is excluded from the control. P9 l 47 The level of significance was set at 95%.. This should be 5% if you mean a significance test or 95% if you are meaning confidence intervals. A significance level of 95% means you reject the null hypothesis when it is true with a probability of 0.95. P10/11 table 1. Can you please state what the unexposed group is in each study. Also I assume that the % in the expose unexposed columns are the % positive for legionella antibodies and that the numbers are number exposed/total number in group. I think that you should explicitly state this. Also – I did not find any mention of study type in this table or in appendix 1. I struggle a bit to see how case control studies are going to give to prevalence and a prevalence ratio P12 l 40-46. I am puzzled by the split into 6 studies with dentists, 2 with students and 2 with assistants as from the definitions in table 1 the two assistants studies also have dentists as well, similarly for the

	students studies. Also 7 studies mention dentists – study 4 seems to have been missed. The reference to this is 3 in the list of references. I am not sure that this paragraph adds a great deal in view of the huge overlap which means you cannot separate the effect for students from that of dentists without some network meta analysis which is probably not justified in view of the comments you make about study quality.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Norman Noah

This is a well-conducted meta-analysis on a difficult subject. The first problem is the limitation of such a small number of studies available that they could work on = 7 or 8 depending on how you count them. Second, there is a very wide variation in findings in these 8 studies - but perhaps that is what a meta-analysis is about. As the authors point out, there is a significant publication bias as well.

• I do not deserve the acknowledgement. [Please ask them to remove it - I have not seen the paper before].

OK

• However two studies used, especially the one from Austria, and also the 'half' one from Bari in Italy, provide results way outside the others. Thus there is not only large variation between countries, but even within countries - e.g the Borella paper from Turin and Bari. Furthermore, LD is influenced by climate, season, and other environmental factors so that nothing is in the end, static. I can't help feeling in the end that one great message of this paper is precisely that: a meta-analysis may be helpful but it is really the responsibility of each country, even every region within a country, to do their own assessments.

Thanks for this important suggestion. In an attempt to generalize the present data to all healthcare workers, particularly hospital staff, we have now reviewed the few papers regarding these categories and drawn speculative conclusions that climatic factors and geographic contexts may be more important than the occupational exposure in explaining anti-Legionella antibody seroprevalence. In the revised version we stole the last referee's sentence and used as conclusion of the Discussion section.

• My only other major point is that the authors should maybe focus more on the difference before and after the CDC guidelines on Infection Control in LD. These were published in 1993, and that authors have very sensibly chosen 1998 as their 'cut-off' point. There is a nice message here that whatever risk there was to dental HCWs before the guidelines, there is some evidence that they have made a difference. They could almost make this as their major objective. It suggests that the risk can be modified substantially.
• All in all, however, the authors make a good case for dental staff not being at great risk of acquiring LD. However possibly vigilance is necessary, as is following good hygiene practices as per existing guidelines from reputable sources.

Thank you very much, we have strengthen this message in the Discussion section in the paragraph where we explained the reasons for the discrepancy between high detection rate of legionellae in

dental unit water systems and lack of occupational risk to dental staff after 1998. We also made it clearer in the Abstract.

Some minor comments

• An overuse of 'among' rather than 'in'.

We have changed some “among” into “in”, particularly when two or more “among” occurred consecutively.

• I am not sure what they mean in the Abstract/Conclusions by including 'the practical contribution of manufacturers' as I do not think this is discussed in the text.

Actually, we did not discuss this issue. Thus, we have deleted the related sentences throughout the text.

• the English, with a few Americanisms thrown in, and some punctuation problems will probably be dealt with by your copy-editors.

We agree that the original version had too many commas, we have removed some of them.

• in the first para of the Introduction, they should give dates as years; this para includes 'Just few years later.....'; 'in the same period.....'; 'a decade later.....'. I was lost long before that last decade!

Very sorry for this!! Indeed, re-reading the Introduction after these months, we agree that following the story of Legionnaires’ disease was difficult.

• the first para of Methods would be better in the Discussion.

Thank you for this suggestion, the para is now the first of the Discussion section.

• towards the end of results, on p 9, they say the 'two subgroup analyses were planned as follows' and then follow this with an incomprehensible paragraph. State exactly what the two groups were after ' as follows'. What these two groups were could be clarified.

We have now tried to clarify the rationale of the subgroup analysis according to healthcare working categories.
However, since the results of this analysis were inconclusive and another Referee did not like it for this reason, we could delete this analysis from the text because it is really not important.

Reviewer: 2
Reviewer Name: Haluk Erdogan

• "Literature search was performed by one Author (SP)" may have potential risk of bias.

Actually, comprehensiveness of literature search may affect the results of a meta-analysis.
Since our last study search update was performed on August 2016 we needed to redo it and, we, to overcome the problem of potential publication/selection bias we did it together (we have now explained it in the text). We, therefore, updated our analysis as it can also be seen from revised Figure 1 and in the first part of the Results section.

• Authors should also focus on the main findings of the study in the discussion section.

Thank you for this comment. We have now added some paragraphs to the Discussion section (added parts are in red characters) dealing with findings and to the generalization of results to all healthcare workers recognizing, however, the limitations of the study.

Reviewer: 3
Reviewer Name: Chris Robertson

This is a very detailed meta-analysis of the risk of carrying legionella antibodies among dental health workers in relation to controls who are occupational but not likely to be exposed to legionella.
I think that this study appears to have been carried out in a careful fashion.
The statistical aspects of the Meta-analysis are standard and are carried out in a very clear and detailed fashion. I think that this is a complex analysis and by and large I think that this work has been well written up.

• It was a little bit difficult for me to understand exactly what had been done in the selection of the studies and this needs to be clarified. In each study selected the prevalence in dental workers is compared to a control group and while there is a list of occupations excluded from the control group, exactly who are the controls in each study were not presented. I think that this is essential and should be listed.

To acknowledge this suggestion and other referee's comments we added a column to Table 1, showing the characteristics of the individuals chosen as occupationally unexposed by the Authors of the various studies, we have also rewritten the "Inclusion and exclusion criteria" subsection.

• Also it was not completely clear to me if the study was just about estimating prevalence, in which case you could have single arm studies, or about comparing prevalence in DHCWs to a control. I concluded that it was the latter but initially was not sure.

We can understand that the choice to include case-ctrl studies, cohort and cross-sectional studies in the list of eligible studies could have sounded confusing to the referee. Now we hope to have provided sufficient details and to have clarified the study design.
At the end of the Intro section that we opted for a relative estimate of the occupational risk and at the beginning of the Methods section the reasons why we preferred a relative risk estimate of the occupational to an absolute risk estimate.

In the Inclusion and exclusion criteria we have clarified that we were looking for analytical cross-sectional studies instead of overall cross-sectional studies.
We changed the title to the "Data extraction" subsection into "Data extraction and outcome". There, we explained that we did not find any cohort studies and case-control studies but only analytical cross-sectional studies and, for this reason, we chose the prevalence ratio as estimate of risk ratio.

More detailed comments

• The pooled PR was statistically non-significant at 95% level (1.7; 95CI, 0.8-3.2),
I do not understand this statement and this that you would be better to clarify that this is the PR comparing exposed DHCWs with unexposed. Also I am not sure what the unexposed group is. Later on you state that all DHCWs are exposed.

One problem with abstract is the 300-word limit. We hope to have provided enough clarifications within the word limit. We, thus, explained the we classified DHCWs as (potentially) occupational exposed, that we found only analytical cross-sectional studies and, therefore, opted for PR as relative occupational risk estimate.

• Page 3 line 11. If the risk is negligible why would you want to ascribe it to anything. I do not really see why you would then attempt to ascribe it to any factor.

Actually, we just wanted to explain why there was a certain occupational risk before 1998 which became negligible after 1998 and, most of all, we wanted to explain the reasons for the apparent contrast with the high frequency of contaminated dental units and the reported negligible occupational risk.
Anyway, we changed "probably" into "possibly" thus decreasing the strength of our speculation.

• P6 line 51. I think you should clarify when case control studies were part of the search as the main aim seems to be to ascertain prevalence of legionella among dental workers. You will get risk from the cohort and cross sectional studies. It has just occurred to me that you might be looking to compare prevalence in different groups of workers and this has not really been clear from the introduction. I think it would be better to make this clear from the start – you are looking for studies where you can compare the prevalence of legionella in DHCWs with another group – At this stage I am not too sure what the other group is – unexposed DHCWs or another unexposed group.

See our comments above.

• Page 6 Inclusion/exclusion criteria.
I am afraid I found these quite difficult to comprehend and think that they need to be expressed in a clearer fashion. 1 is fine but 2-5 are not really inclusion/exclusion criteria for studies. I think that the rest mean that within each study you have to have sufficient information to be able to identify a group of exposed DHCWs and a controls group of workers who are not DHCWs and who are not likely to be exposed to legionella through their jobs

We have tried to simplify this section, with an introductory paragraph explaining the concepts of occupational and non-occupational exposure to Legionella inhalation. In addition, in item #1 we have explained that we sought for analytical cross-sectional studies. We have also merged items 2 and 3

which actually refer to the same idea of identifying dental healthcare workers. We also removed the category of controls and changed it into “occupationally unexposed individuals”, which is more correct, thus making it clearer that these subjects could be exposed to the risk of Legionella infection during their life but not because of their jobs. We also changed the last item, from “outcome” to “Legionella infection” and explained what criteria we accepted to consider individuals as infected with legionellae.

• P 6 l 19-35 please state explicitly why each occupational group is excluded from the control.

We have added a paragraph at the beginning of the Inclusion and exclusion criteria subsection explaining how occupational and non-occupational exposure to Legionella occurs. On the basis of this paragraph we then listed in new item #3 the occupational categories which could not be considered as occupationally unexposed. We also included a recent review on LD cases among occupationally exposed individuals, thus strengthening the reported sentences.

• P9 l 47 The level of significance was set at 95%.. This should be 5% if you mean a significance test or 95% if you are meaning confidence intervals. A significance level of 95% means you reject the null hypothesis when it is true with a probability of 0.95.

Thank you for this important suggestion and sorry for our oversight! Significance level of statistical tests is… now 0.05!
:-/

• P10/11 table 1. Can you please state what the unexposed group is in each study. Also I assume that the % in the expose unexposed columns are the % positive for legionella antibodies and that the numbers are number exposed/total number in group. I think that you should explicitly state this.

Thank you for this suggestion. Now we have added another column to Table 1 and corrected the headings of the columns reporting prevalence rates.

• Also – I did not find any mention of study type in this table or in appendix 1. I struggle a bit to see how case control studies are going to give to prevalence and a prevalence ratio

Now we have explained in Abstract, Results and also anticipated in the Methods section that we did not find case-ctrl and cohort studies, but that all studies were analytical cross-sectional. This was actually important to understand our choice to opt for prevalence ratio as outcome measure of relative occupational risk.

• P12 l 40-46. I am puzzled by the split into 6 studies with dentists, 2 with students and 2 with assistants as from the definitions in table 1 the two assistants studies also have dentists as well, similarly for the students studies. Also 7 studies mention dentists – study 4 seems to have been missed. The reference to this is 3 in the list of references. I am not sure that this paragraph adds a great deal in view of the huge overlap which means you cannot separate the effect for students from that of dentists without some network meta-analysis which is probably not justified in view of the comments you make about study quality.

We did not include the study of Fotos and colleagues in this subgroup analysis because the sample of

dental healthcare workers was heterogeneous and specific data for each category were not reported. Now we have provided an explanation for the choice of performing a subgroup analysis according to working categories. In the event we detected an important occupational risk in dental healthcare settings, differences between working categories could help explain the between-study heterogeneity. However, there results were inconclusive and we included a sentence on this in the Abstract. Anyway, upon referee's request, we are ready to delete this analysis.

VERSION 2 – REVIEW

REVIEWER	Norman Noah LSHTM, London, UK
REVIEW RETURNED	21-Mar-2017

GENERAL COMMENTS	This paper has certainly improved but I think the authors need to stress more some of the limitations of it – thus in fact and somewhat paradoxically, strengthening the paper. These studies collectively included 2,232 dental healthcare workers and 1,172 occupationally unexposed individuals. Screening provided thirty-two papers, eighteen of them immediately excluded. Of the remaining fourteen studies, seven were then further excluded. This left in the end only 7/8 studies. Disappointing but not that unusual in meta-analyses, and not in itself necessarily a weakness. 2,232 dental healthcare workers and 1,172 occupationally unexposed individuals seem like large numbers, but one also has to consider the number of studies, especially if the meta-analysis covers only a small number of countries. This gives a less confident appraisal of the total burden of LD in this occupational group, as reflected in the huge variation in findings. With such a vast difference in the prevalence ratios in table 1 spread over a relatively small number of studies, pooling the overall prevalence is a little artificial and possibly meaningless. Indeed two studies were so different from the others – making it difficult to interpret when there were only 8 studies to start with. There was also considerable variation in the actual rates of positives – 0.4% to 33.6%, which I think should be commented upon. It was reassuring to be told that the weight of three studies 3,8,51 accounted for almost three fourths of the overall weight. However, the exclusion of these studies in turn did not produce a significant change in the pooled prevalence ratio estimate. Perhaps this ought to be expanded with some data to corroborate it. It follows from this that the message of this paper, as I commented earlier, is that the prevalence ratios in each country depend on a very large number of factors: some that come to mind are the representativeness of the samples studied in each country, the accuracy of the serological tests, possibly the completeness of the surveillance, the background rate of positive legionella serology (which is not the same as disease), and indeed how diligently they have implemented the CDC guidelines. Thus I think the authors need to stress even more than they do in this revised version, how virtually all of this depends on local conditions, as stated in the previous sentence. There is wide variation which needs to be explained and perhaps the main importance of this paper is to draw attention to this variation, and for countries to address the same questions of occupational LD from dental offices.

	Some minor comments: Introduction “Nevertheless, several reviews, editorials and discussion papers warn that the occupational risk of Legionella infection to the dental staff is high (references 10,16,17 are only recent examples).” The papers describing low risk are given more space than these apparently high risk ones – it would be helpful to the reader to deal with these in the same way as they have dealt with refs 9-12 earlier in the paragraph. ‘dental infections’ Reads like what it says – they mean infections acquired after dentistry or associated with dentistry, not tooth infections. Insert ‘ local’ before ‘climatic and geographic characteristics’ in Strengths And Limitations “thus making it impossible to discern which of the two systems was the source of infection and which was contaminated by the diseased dentist”. How could the dentist have contaminated the water? LD is never human in origin. Much of the English still needs attention; a few examples - associated to; associable; the majority of = most; practicing. ‘hypothesis’ may be a better word to use than ‘speculation’. Table 1 – include reference numbers to the 8 papers mentioned.

REVIEWER	Erdoğan, Haluk Baskent University, Faculty of Medicine, Department of Infectious Disease and Clinical Microbiology, Turkey.
REVIEW RETURNED	16-Mar-2017

GENERAL COMMENTS	The authors have corrected the manuscript according to my suggestion so the manuscript is acceptable.

VERSION 2 – AUTHOR RESPONSE

Thank you very much for your comments, we think that they were very useful to improve the quality and the clarity of our meta-analysis. We have re-revised our manuscript according to Reviewer #2 comments further stressing its limitations as requested as follows.

We have emphasized several times in the text that the results of the present meta-analysis in occupational epidemiology must be interpreted with caution and considered as an indication, rather than a measure of the occupational risk.
We also deleted the sentence that the occupational risk is negligible (which implies that there is evidence of no risk) and changed it into a softer sentence, namely, that there is limited scientific evidence of occupational risk. This sentence implies that the primary studies do not allow to provide a definitive response.
Finally, we have tried to provide an explanation to the differences between studies in both absolute prevalence and in prevalence ratio. We also wrote that this explanation was only a conjecture and that a final answer whether healthcare workers are at occupational risk of Legionella infection is provided by cohort studies which assess exposure (i.e., Legionella loads in artificial water systems) and outcome (i.e., Legionella antibody seroconversion). We have added three more paragraphs in the

Discussion section.

We also acknowledged the minor comments and suggestions as follows,

Reviewer
Introduction
“Nevertheless, several reviews, editorials and discussion papers warn that the occupational risk of Legionella infection to the dental staff is high (references 10,16,17 are only recent examples).” The papers describing low risk are given more space than these apparently high risk ones – it would be helpful to the reader to deal with these in the same way as they have dealt with refs 9-12 earlier in the paragraph.
Authors
In this re-revised version we have explained why these Authors considered the occupational risk for Legionella infection to dental staff high with a list of four arguments. We had already reported these arguments in previous paragraphs, but now we have inserted a specific paragraph listing them. We also added another paragraph explaining why some of these arguments were not convincing. We needed to do this because this was the justification of the present meta-analysis.

Reviewer
‘dental infections’ Reads like what it says – they mean infections acquired after dentistry or associated with dentistry, not tooth infections.
Authors
Dental infections are the words used by Holmes and colleagues “three [out of 30 patients with LD] had had preceding dental infections”. If patients with LD had past dental infections perhaps they sought for dental healthcare. We have now added this sentence. Of course the Authors at that time had no idea regarding LD transmission. For example, they also reported that “four patients had been exposed to birds”.

Reviewer
Insert ‘ local’ before ‘climatic and geographic characteristics’ in Strengths And Limitations
Authors
OK

Reviewer
“thus making it impossible to discern which of the two systems was the source of infection and which was contaminated by the diseased dentist”. How could the dentist have contaminated the water? LD is never human in origin.
Authors
The recent episode of a man who acquired Legionnaires’ Disease 300 Km away from home and then, back to home, infected her mother, while she was taking care of her after just 8 hours of contact, made it clear that human-to-human infection transmission is possible and that infected patients can spread legionellae in the environment. This, by the way, may explain those apparently astonishing cases of LD developed up to ten kilometers away from the cooling towers that acted as source of outbreak. We have now explained this in the Intro section.

Reviewer
Much of the English still needs attention; a few examples - associated to; associable; the majority of = most; practicing.
Authors
We have now edited our manuscript

Reviewer
'hypothesis' may be a better word to use than 'speculation'.
Authors
OK

Reviewer
Table 1 – include reference numbers to the 8 papers mentioned.
Authors
OK

Again, thank you very much for your time and the excellent suggestions.

VERSION 3 – REVIEW

REVIEWER	Norman Noah LSHTM London, UK
REVIEW RETURNED	04-Jun-2017

GENERAL COMMENTS	I have no further comments on this paper. The authors have strengthened the limitations adequately.