PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Enteric Salmonella in humans and food in the Middle East and North Africa: Protocol of a systematic review
AUTHORS	Chaabna, Karima; Alali, Walid

VERSION 1 – REVIEW

REVIEWER	Dr Andrea Parisi Australian National University
REVIEW RETURNED	22-Oct-2016

GENERAL COMMENTS	The manuscript is nicely written but it is not very interesting for the reader to read as it is not going to have any significant impact on public health. It is riddled with grammatical mistakes ("this protocol provides methods will be used"...) and spelling errors ("Te World Health Organization", "preform"). It lacked sufficient detail for me to understand how it worked. It didn't follow PRISMA checklist as the search strategy wasn't clearly defined (missing primary and secondary search terms etc.). I believe that it is not appropriate for BMJ Open as it is not very informative (it would be better if it had final outputs).

REVIEWER	Sara Pires National Food Institute, Technical University of Denmark
REVIEW RETURNED	02-Nov-2016

GENERAL COMMENTS	Manuscript review: bmjopen-2016-014411 "Enteric Salmonella in humans and food in the Middle East and North Africa: Protocol of a systematic review". This is a well written and structured protocol for a systematic review (SR) that aims at collecting human and food Salmonella data in the MENA region. The data to be collected will address an important data gap, and will allow for the conduction of studies that will be crucial to provide evidence to inform food safety strategies at a national and regional level. I have few general comments and some more detailed specific comments. General comments: - The manuscript describes a protocol for an important and useful study. However, it is unclear if the aim is to describe and share (i.e. publish) a protocol that can be used by any researcher wanting to conduct such a SR, of for the authors' study purpose. The latter would make sense, since a comprehensive SR would be able to capture all relevant data and would not need to be replicated for a period of time. In this case, why would the authors publish the protocol and the study's details and results separately?

	- The data that the described SR aims to compile would be very useful for source attribution studies at the regional and national level. Source attribution partitions human cases of disease to the most important sources (which can be foods, animals), estimating the relative contribution of these. Such evidence is determinant to identify and prioritize food safety interventions. These data are generally lacking in the region, and so this would be a great value of the SR and should be described in the manuscript. Specific comments: Abstract: Page 2, line 10: “methods will be used” should be replaced by “methods that will be used”. Strengths and limitations of this study: - It would also be important to mention that heterogeneity due to different sample sizes in food studies is possible/expected. Introduction: Page 4, line 15: Does it make sense to site US estimates only (and not other countries which also estimated burden of salmonellosis at a national level)? Being a country so different from countries in the MENA region, maybe the authors could refer just WHO-FERG's estimates. Objectives: Page 4, lines 29 and onwards: It would also be relevant to mention that compiling data from human cases and prevalence in foods in a given population/geographic area will allow for an estimation of the most important sources of disease in the population, as well as their relative contribution (source attribution). These estimates will be crucial to identify and prioritize food safety interventions in countries. Type of studies: Page 4, lines 48 onwards: Is this only for food prevalence studies? I imagine that for human cases (unless it's a community study) there is actually no sample size? And why 10? Types of exposures: Page 5, line 15: Linking to my comment above, it is apparent that human data will be from outpatients and inpatients, and thus a sample size doesn't apply. Selection procedure Page 6, line 7: IS there a need for two (or more) reviewers, and to discuss potential conflicts in exclusion and inclusion of studies according to the defined criteria? Data synthesis Page 6, line 54: So there will be community studies? This should be described above. Page 6, line 56 (point 2): Suspected - I suppose a diagnostic is only possible when the agent is identified at the lab. Page 7, lines 3-5: I think this is an important outcome and should be described. Prevalence (with number of isolates and sources) of different Salmonella subtypes in different foods (per study) would be very useful data. In this respect, it would be useful to have a harmonized food categorization scheme. Page 7, line 7: I didn't understand what the outcome of the meta-analisis would be. Would it be an incidence of salmonella in a country, combining data from multiple studies? Or prevalence in a given food type? This is not clear in the protocol.

	Discussion: Page 7, line 47: attempting should be replaced by attempt. Page 7, lines 47-57: As referred above, I believe it would be useful to discuss the utility of such data for the purpose of source attribution. Several studies have attributed human salmonellosis cases to sources by linking Salmonella subtyping data in human cases and in foods or animals, and these estimates have proven useful for risk management. Such studies have been applied in Denmark, EU-level, the Netherlands, France, US, among others.

REVIEWER	Iruka N Okeke University of Ibadan, Nigeria
REVIEW RETURNED	09-Jan-2017

GENERAL COMMENTS	The burden of disease, clinical consequences and sequelae from Salmonella infections are inadequately understood, particularly in Africa, South America and the Middle East where culture of clinical, food, animal and environmental isolates is uncommon. The authors plan a systematic review of available data on non-typhoidal Salmonella (NTS) from humans and food samples in North Africa and the Middle East. This is a needed study and the design is largely appropriate. The investigators plan to adhere to PRISMA guidelines and have based the protocol on the Cochrane handbook. I don't have technical expertise in metanalysis and can therefore not review that portion of the manuscript. I have a few minor concerns, which if addressed could improve the quality of the systematic review. There are a dozen different right ways (and perhaps several-fold more wrong ones) to recover, identify and confirm Salmonella in food and clinical specimens. When enrichment is used, the yield of Salmonella will go up but not all studies use pre-enrichment. Once presumptive Salmonella are obtained, ruling out related species requires rigorous identification protocols. Serotyping biochemically confirmed Salmonella is the current Gold standard for identification but serotyping results depend on preliminary testing methods that are used to eliminate cross-reacting strains, the quality and age of antisera used as well as the skill of the technician. These can all be assured at reference centers but if non-reference center data is uniformly excluded, most of the papers obtained will not be included in the study. How do the investigators plan to evaluate the quality of laboratory methods used? Will they at least require biochemical verification and commercially sourced antisera? Whatever they decide, it may be wise to include acceptable criteria for laboratory verification in the protocol. This would be superfluous for many pathogens but is useful for Salmonella. Molecular methods are much less plagued by the methodological limitations of serotyping but are unavailable in many African laboratories. Can the investigators note what proportion of studies were based on molecular identification and confirmation? I wonder whether some articles will fall between the cracks just because some invasive NTS infections have a foodborne origin. Additionally, prior to very recent identification of ST313 and other invasive NTS lineages, the distinction between those strains and intestinal NTS would be difficult to parse. Unless it results in a very

	large number of typhoid papers, it might be easier to review all Salmonellosis at once. The authors should give this some thought. If the authors choose not to do this, then they should include this as a limitation of the study The paper has a few typographical errors. For example, one more words is missing from page 2 line 10.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Dr Andrea Parisi

Institution and Country: Australian National University

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The manuscript is nicely written but it is not very interesting for the reader to read as it is not going to have any significant impact on public health.

Response: The proposed manuscript is a protocol for an ongoing systematic review aims to describe *Salmonella* infection epidemiology in the Middle East and North Africa Region. The specific objective of the protocol is to provide detailed stand-alone methodology that will allow the readers to use for similar systematic reviews. Furthermore, it will be used as a reference when we publish our systematic review and meta-analysis findings; given the limited word count attributed to the method section of a systematic review manuscript. Moreover, according to the PRISMA guidelines, transparent reporting of a systematic review methodology is a must. Additionally, we would like to obtain feedback on our study protocol through the peer review process, and to prevent both "data dredging" as well as minimizing bias by explicitly stating *priori* hypotheses and methods without prior knowledge of results. Moreover, a published systematic review protocol will avoid duplication of research effort carried by other investigators.

It is riddled with grammatical mistakes ("this protocol provides methods will be used"...) and spelling errors ("Te World Health Organization", "preform").

Response: We thank the reviewer for the comment. We have carefully revised the manuscript for grammatical and spelling errors.

It lacked sufficient detail for me to understand how it worked.

Response: We followed the latest standards in describing methods of systematic review and meta-analysis and we presented them in details following PRISMA-P guidelines. However, we have provided more in depth details on how the search criteria was built and what are the countries of the MENA region we want to study (page 6, lines 8-20).

It didn't follow PRISMA checklist as the search strategy wasn't clearly defined (missing primary and secondary search terms etc.).

Response: PRISMA checklist is a tool that helps researchers to report their systematic reviews. As the proposed manuscript is a protocol of a systematic review, we followed PRISMA-P check list. To address the reviewer comment we have now stated that we will report our systematic review following

PRISMA 2009 statements and PRISMA for Abstracts Checklist (page 7, lines 16-17). Additionally, we now provided more details regarding the search strategy (page 6, lines 8-19).

I believe that it is not appropriate for BMJ Open as it is not very informative (it would be better if it had final outputs).

Response: Please see our earlier responses (above) to the reviewer's comments. We do believe that our protocol is appropriate for BMJ Open. BMJ Open is a journal that publishes protocol of systematic review. Like BMJ Open, we believe that the benefits of publishing a systematic review protocol on its own are high and would greatly improve the systematic review and meta-analysis findings and reporting.

Reviewer: 2

Reviewer Name: Sara Pires

Institution and Country: National Food Institute, Technical University of Denmark

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

Manuscript review: bmjopen-2016-014411 "Enteric Salmonella in humans and food in the Middle East and North Africa: Protocol of a systematic review".

This is a well written and structured protocol for a systematic review (SR) that aims at collecting human and food Salmonella data in the MENA region. The data to be collected will address an important data gap, and will allow for the conduction of studies that will be crucial to provide evidence to inform food safety strategies at a national and regional level. I have few general comments and some more detailed specific comments.

General comments:

- *The manuscript describes a protocol for an important and useful study. However, it is unclear if the aim is to describe and share (i.e. publish) a protocol that can be used by any researcher wanting to conduct such a SR, of for the authors' study purpose. The latter would make sense, since a comprehensive SR would be able to capture all relevant data and would not need to be replicated for a period of time. In this case, why would the authors publish the protocol and the study's details and results separately?*

Response:

The proposed manuscript is a protocol for an ongoing systematic review aims to describe *Salmonella* infection epidemiology in the Middle East and North Africa Region. The objective of the protocol is to provide detailed stand-alone methodology that will allow the readers to use for similar systematic reviews. Furthermore, it will be used as a reference when we publish our systematic review and meta-analysis findings; given the limited word count attributed to the method section of a systematic review manuscript. According to the PRISMA guidelines, transparent reporting of a systematic review methodology is a must. Additionally, we would like to obtain feedback on our study protocol through the peer review process, and to prevent both "data dredging" as well as minimizing bias by explicitly stating *a priori* hypotheses and methods without prior knowledge of results. Moreover, a published systematic review protocol will avoid duplication of research effort carried by other investigators.

- *The data that the described SR aims to compile would be very useful for source attribution studies at the regional and national level. Source attribution partitions human cases of disease to the most important sources (which can be foods, animals), estimating the relative contribution of these. Such evidence is determinant to identify and prioritize food safety interventions. These data are generally lacking in the region, and so this would be a great value of the SR and should be described in the manuscript.*

Response: We very much agree with the reviewer that this systematic review findings (NTS prevalence in humans and in food) can be used by the region's countries for their risk assessment, research priorities, and development of science-based food safety interventions. We addressed this comment in the manuscript according to the reviewer's comment (page 4, line 21-25)

Specific comments:

Abstract:

Page 2, line 10: "methods will be used" should be replaced by "methods that will be used".

Response: Change has been made in the manuscript text (page 2, line 4)

Strengths and limitations of this study:

- *It would also be important to mention that heterogeneity due to different sample sizes in food studies is possible/expected.*

Response*:* We agree with the reviewer and we addressed this comment in the manuscript (page 3, lines 7-8 and page 7, lines 28).

Introduction:

Page 4, line 15: Does it make sense to site US estimates only (and not other countries which also estimated burden of salmonellosis at a national level)? Being a country so different from countries in the MENA region, maybe the authors could refer just WHO-FERG's estimates.

Response*:* In fact we provided WHO global and region specific estimates in the introduction, and not only the U.S. estimates. *"The World Health Organization (WHO) estimated that the annual median number of nontyphoidal salmonellosis was 78.7 million foodborne illnesses with over 59 thousand deaths. As for the WHO defined Eastern Mediterranean Region, the median incidence rate of nontyphoidal salmonellosis was 1,610 illnesses with 0.6 death, and 54 disability adjusted life years (DALYS) per 100,000 persons; whereas, the median incidence rate in the WHO defined African Region is 896 illnesses with 1 death, and 89 DALYS per 100,000 persons".* Unfortunately, country-specific estimates are not available.

Objectives:

Page 4, lines 29 and onwards: It would also be relevant to mention that compiling data from human cases and prevalence in foods in a given population/geographic area will allow for an estimation of the most important sources of disease in the population, as well as their relative contribution (source attribution). These estimates will be crucial to identify and prioritize food safety interventions in countries.

Response: We agree with the reviewer. This has been added to the manuscript text (page 4, lines 21-25 and page 8, lines 22-25)

Type of studies:

Page 4, lines 48 onwards: Is this only for food prevalence studies? I imagine that for human cases (unless it's a community study) there is actually no sample size? And why 10?

Response: We aim to estimate the pooled prevalence of *Salmonella* in food and in human, not the number of positive cases. In order to estimate our pooled prevalence of *Salmonella* in food and in human, we will include only studies reporting the number of *Salmonella* positive cases and the population size that gave rise to cases.

Regarding the sample size of 10, we want to be most inclusive. However, we will recalculate the minimum sample size for a 'good' precision to include those studies with a sample size higher than this minimum sample size in our meta-analysis.

Types of exposures:

Page 5, line 15: Linking to my comment above, it is apparent that human data will be from outpatients and inpatients, and thus a sample size doesn't apply.

Response: We addressed this comment in the response (above) regarding the sample size.

Selection procedure

Page 6, line 7: IS there a need for two (or more) reviewers, and to discuss potential conflicts in exclusion and inclusion of studies according to the defined criteria?

Response: We have clarified our selection process in the manuscript text as follows: The title and abstract screening for relevance, followed by the full-text screening of the unique reports will be conducted by KC. All this multi-level screening process will be checked by WA. Any disagreements will be resolved by discussion and consensus (page 6, lines 24-27).

Data synthesis

Page 6, line 54: So there will be community studies? This should be described above.

Response: We will include any observational study reporting prevalence (%) with the number of positive cases identified at laboratories and the corresponding sample size. The setting of these studies can be community or clinical. We have clarified that in the manuscript (page 5, line 8; and page 7, lines 3-4 and line 20)

Page 6, line 56 (point 2): Suspected - I suppose a diagnostic is only possible when the agent is identified at the lab.

Response: We will include only studies reporting the number of positive cases that have been identified at laboratories.

Page 7, lines 3-5: I think this is an important outcome and should be described. Prevalence (with number of isolates and sources) of different Salmonella subtypes in different foods (per study) would be very useful data. In this respect, it would be useful to have a harmonized food categorization scheme.

Response: Food will be categorized into the following groups: poultry, beef, seafood, dairy, complex food, among others. We have clarified that in the manuscript (page 7 line 26).

Page 7, line 7: I didn't understand what the outcome of the meta-analisis would be. Would it be a 5n incidence of salmonella in a country, combining data from multiple studies? Or prevalence in a given food type? This is not clear in the protocol.

Response: Using meta-analysis, we aim to estimate pooled prevalence of *Salmonella* in food (stratified by category) and in human (stratified by type of population). (page 7, lines 25-26)

Discussion:

Page 7, line 47: attempting should be replaced by attempt.

Response: Change has been made to the text (page 8, line 18)

Page 7, lines 47-57: As referred above, I believe it would be useful to discuss the utility of such data for the purpose of source attribution. Several studies have attributed human salmonellosis cases to sources by linking Salmonella subtyping data in human cases and in foods or animals, and these estimates have proven useful for risk management. Such studies have been applied in Denmark, EU-level, the Netherlands, France, US, among others.

Response: We have mentioned that in the introduction, objectives, and now have been added it to the discussion section (page 4, lines 21-25 and page 8, lines 22-25).

Reviewer: 3

Reviewer Name: Iruka N Okeke

Institution and Country: University of Ibadan, Nigeria

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The burden of disease, clinical consequences and sequelae from Salmonella infections are inadequately understood, particularly in Africa, South America and the Middle East where culture of clinical, food, animal and environmental isolates is uncommon. The authors plan a systematic review of available data on non-typhoidal Salmonella (NTS) from humans and food samples in North Africa and the Middle East. This is a needed study and the design is largely appropriate. The investigators plan to adhere to PRISMA guidelines and have based the protocol on the Cochrane handbook. I don't have technical expertise in metanalysis and can therefore not review that portion of the manuscript. I have a few minor concerns, which if addressed could improve the quality of the systematic review.

There are a dozen different right ways (and perhaps several-fold more wrong ones) to recover, identify and confirm Salmonella in food and clinical specimens. When enrichment is used, the yield of Salmonella will go up but not all studies use pre-enrichment. Once presumptive Salmonella are obtained, ruling out related species requires rigorous identification protocols. Serotyping biochemically confirmed Salmonella is the current Gold standard for identification but serotyping results depend on preliminary testing methods that are used to eliminate cross-reacting strains, the quality and age of antisera used as well as the skill of the technician. These can all be assured at reference centers but if non-reference center data is uniformly excluded, most of the papers obtained will not be included in the study. How do the investigators plan to evaluate the quality of laboratory methods used? Will they at least require biochemical verification and commercially sourced antisera? Whatever they decide, it may be wise to include acceptable criteria for laboratory verification in the protocol. This would be superfluous for many pathogens but is useful for Salmonella.

Response*:* We will include studies that used conventional methods to culture *Salmonella* (with pre-enrichment) and with or without PCR method for *Salmonella* confirmation. As for serotyping, we will include studies that used commercially known antisera (supplied by BD, Oxoid, and Bio-Rad).

Molecular methods are much less plagued by the methodological limitations of serotyping but are unavailable in many African laboratories. Can the investigators note what proportion of studies were based on molecular identification and confirmation?

Response: Since this is a protocol for a systematic review that is ongoing, we do not have yet the proportion of studies that are based on molecular identification and confirmation. However, we will address the reviewer question in our systematic review manuscript.

I wonder whether some articles will fall between the cracks just because some invasive NTS infections have a foodborne origin. Additionally, prior to very recent identification of ST313 and other invasive NTS lineages, the distinction between those strains and intestinal NTS would be difficult to parse. Unless it results in a very large number of typhoid papers, it might be easier to review all Salmonellosis at once. The authors should give this some thought. If the authors choose not to do this, then they should include this as a limitation of the study

Response: We have very few studies from the MENA region that reported invasive non-typhoidal *Salmonella*. Hence, these studies will be excluded because they did not report a gastrointestinal enteritis nor a foodborne link. This has been clarified in the manuscript.

The paper has a few typographical errors. For example, one more words is missing from page 2 line 10.

Response*:* We have carefully revised the manuscript for grammatical and spelling errors.

VERSION 2 – REVIEW

REVIEWER	Sara Pires National Food Institute, Technical University of Denmark
REVIEW RETURNED	15-May-2017

GENERAL COMMENTS	The protocol has greatly improved and is very well written. I have only very few and minor comments: Page 1: Line 6: DALYs are not incidence, but burden of disease Line 8, 9: I would still suggest removing the US estimate – there are many other estimates from individual countries (even if not from the MENA region) Page 8, line 32: replace “sources attribution” by “source attribution”. I also suggest adding a reference for this statement and others in the discussion.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Sara Pires
Institution and Country: National Food Institute, Technical University of Denmark
Please state any competing interests or state 'None declared': None declared
Please leave your comments for the authors below
The protocol has greatly improved and is very well written. I have only very few and minor comments:

Page 1:
Line 6: DALYs are not incidence, but burden of disease
Response: We agree with Reviewer1. We updated our statement in the introduction section (line 6 page 4)

Line 8, 9: I would still suggest removing the US estimate – there are many other estimates from individual countries (even if not from the MENA region)
Response: We cite now estimates in WHO Eastern Mediterranean region and in WHO African region (lines 4-8 page 4) and we have removed the US estimates (lines 8-10 page 4).

Page 8, line 32: replace "sources attribution" by "source attribution". I also suggest adding a reference for this statement and others in the discussion.
Response: We have corrected our protocol (line 24 page 4 and line 23 page 8) and added a reference for this statement in the discussion (line 24 page 8)