PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Burden of seasonal influenza in sub Saharan Africa: A systematic review protocol
AUTHORS	Sambala, Evanson; Mdolo, Aaron; Banda, Richard; Phiri, Arthur; Wiyeh, Alison; Wiysonge, Charles

VERSION 1 – REVIEW

REVIEWER	Prof Harish Nair University of Edinburgh
REVIEW RETURNED	27-Mar-2018

GENERAL COMMENTS	1. Could you please clarify how you would deal with studies that include data where study duration overlaps in part the "pandemic period" ? 2. Please clearly define the denominators for mortality rate, attack rate, hospitalisation rate. The way you now define hospitalisation rate is not correct- you are assessing the proportion of hospitalisations that were due to seasonal flu (proportion is not rate!). 3. Prevalence rate- clarify if you are looking at point prevalence or period prevalence? 4. Clarify if you are interested in lab confirmed flu only? How do you deal with ILI and ICD coded studies where you are unsure of lab status? 5. What specific age bands do you wish to report? 6. Do you plan to run the same search strategy across the different databases?

REVIEWER	Nita K. Madhav, Head of Data Science Metabiota, USA
REVIEW RETURNED	30-May-2018

GENERAL COMMENTS	The authors have selected an area of urgent research for influenza. The goal of assessing the influenza burden in sub-Saharan Africa is certainly an area requiring further study. However, the protocol as presented leaves unanswered some key questions regarding the methods that will be used to undertake this study. Below, please find elaboration to the "No" answers to the Review questions indicated above: 4. Are the methods described sufficiently to allow the study to be repeated? While the methods provided for the systematic review of literature appear to be generally sound, the description of the anticipated

	methods for the meta-analysis requires further clarification. Especially, the authors should discuss the methods they will use to reconcile different case definitions, reporting thresholds, and reporting systems. The studies that are found may contain different outcome measures such as influenza-like illness (ILI), acute respiratory illness (ARI), severe acute respiratory illness (SARI), pneumonia, etc. It is not clear from the protocol, as written, how these disparate types of information will be assessed, transformed, and/or processed for combinability in the meta-analysis. Furthermore, the authors indicated they would include “seasonal influenza virus infections”, but the authors have not specified the case definition that will be used. For example, the case definition might be very narrow (e.g., will only include cases having PCR laboratory confirmation) or might be very broad (“influenza-like illnesses"). Further clarification on the case definition should be provided. The statement “Our search will not place any search limitation on the outcome of the burdens of seasonal influenza or date except for the study location.” Seems to be at odds with the statement earlier in the document, where it was indicated that the authors would be excluding data from the pandemic of 2009-2010. 6. Are the outcomes clearly defined? As indicated in the response to Question 4, the outcomes should be more clearly defined. Additionally, it is unclear how the "population at risk" will be assessed for the incidence rate. This will be especially important for seasonal influenza due to potential pre-existing and cross immunity. There should be some discussion of how this will be considered as well. Also, the definitions of “mortality rate” and "prevalence rate" as presented is incorrect. These should both be defined as proportions, with the numerators and denominators clearly described. 7. If statistics are used are they appropriate and described fully? The statement "We anticipate a wide variation in the identified results as such pooled data will be tested for heterogeneity and non-heterogeneity by deliberately dropping one study with the most variability one at a time from the analyses.” Requires further clarification, as it is not clear what this means or the intention of this analysis. It is also not clear from the description of the analysis what study characteristics will be included as covariates in the meta-analysis (for example, whether the study was active or passive, the income category of the country, etc.). There should be more discussion around the anticipated covariates. 12. Are the study limitations discussed adequately? The biggest limitation that the authors have not appeared to address is the disparity in influenza burden reporting and publication that would lead to a high chance of non-combinability between studies. The mitigation approaches that the authors plan to take against this potential limitation should be discussed in more detail.

	In conclusion, for the reasons stated above, I believe a major revision is required. However, based on the content provided in the protocol, I also believe that the authors are up to the task, and I look forward to reviewing further revisions.

REVIEWER	Gideon Emukule CDC-Kenya Country Office.
REVIEW RETURNED	04-Jun-2018

GENERAL COMMENTS	This review aims to investigate the epidemiological burden of seasonal influenza and highlight its epidemiological patterns in sub Saharan African countries and its findings will contribute to the better understanding of the burden of seasonal influenza. Comments: 1. In the abstract (page 2) the authors refer to using “a Meta command”. I suggest they change this to read “a meta-analysis”. 2. In the introduction (page 4) the authors refer to only two groups, children <5 and the elderly as those most commonly affected by influenza-related complications. They could edit this to also include pregnant women and persons with chronic medical conditions. 3. On page 5, the authors state that “Seasonal influenza activity in the tropical region is not strictly seasonal as it occurs throughout the year, with bi-seasonal peaks in summer and winter”. Can they be more explicit as to when exactly “summer” and “winter” are in the context of sub-Saharan Africa where they intend to conduct the study? 4. On page 6, the authors state that “Studies reporting the burden in age clusters not representative of these subpopulation will be excluded.” Could they state, upfront, what age clusters they plan to consider in their analysis? 5. On page 7, the authors state “We will exclude economic studies that report burden indicators such as financial costs associated to influenza, quality-adjusted life years (QALYs) or disability-adjusted life years (DALYs)”. Is there any particular reason for them excluding economic burden data from the review? If so, can they state it here? 6. On page 7, the authors state “The titles searched by hand will be retained into the database search for a full text.” This sentence seems to be incomplete. Can they rephrase? 7. On page 8 where the authors state “a Meta command”, I suggest they state “meta-analysis to aggregate estimate measures” or better still properly refer to the “metan command” in stata if that is the intention. 8. On page 10, the last sentence that reads “It can also be a predictive indicator to aid estimates of additional capacities that may be may needed to deal with a new pandemic activity in an event it occurs” is not very clear. Could the authors consider to rephrase?

VERSION 1 – AUTHOR RESPONSE

Responses to reviewer comments

We thank the reviewers (Professors Harish Nair, Nita K. Madhav and Gideon Emukule) for critically engaging with our manuscript, and for providing such extremely helpful and detailed comments. Thank you for the opportunity to amend the protocol and resubmit. We have considered the comments and made substantial changes to the protocol. We have maintained the core structure of the protocol and focused the revision specifically on the issues raised by the reviewers in each section of the proposed protocol. We have addressed each reviewers comments point by point and taken the opportunity when revising this protocol to sharpen the writing and correct occasional typographic and other errors. The page numbers cited in our responses refer to the clean version of the manuscript.	
Comments	**Responses**
Reviewer 1	
Could you please clarify how you would deal with studies that include data where study duration overlaps in part the "pandemic period" ?	This has been clarified under the types of studies to be included. We will exclude overlapping studies or data that covers the pandemic period (April, 2009- August, 2010). This has been amended as follows in the manuscript “We will exclude all studies that use data reported between 31 January 2009 and 1 November 2010 including all studies where study duration overlaps or combines the pandemic and non pandemic- periods”
Please clearly define the denominators for mortality rate, attack rate, hospitalization rate. The way you now define hospitalization rate is not correct- you are assessing the proportion of hospitalizations that were due to seasonal flu (proportion is not rate!).	Thank you for pointing out errors in our definitions. We have now incorporated denominators and our definitions are as follows: Mortality rate is defined as the number of deaths in a specific age group due to seasonal influenza, divided by population of age group expressed in 1,000 person years. Attack rate refers to number of new cases of seasonal influenza disease during specified time interval, divided by the number of persons or age groups at risk in the population at start of time interval. Hospitalization rate is a number of influenza inpatient admissions over a specific time and geographical area divided by the population in that age group, expressed in terms of 1000 people days. Incidence rate is the number of new cases during a specified time per population at risk in a given time period whereas period prevalence rate is a measurement of new and

	preexisting of all individuals affected by the disease over a specified period of time divided by total number of people in that population. Page 7, first paragraph, line 5-14 We further defined our outcomes as follows “If burden estimates are reported by week or month, we will calculate yearly burden estimates based on methods provided in the WHO manual for estimating disease burden associated with seasonal influenza. Where studies provide relevant data for the catchment population, we will pool burden proportions of all cases sampled among SARI cases from whom clinical specimen were tested by week/month/year by dividing the total number of SARI cases by month/week/year and multiplying it by 100%. We will adjust for true total number of influenza-associated SARI cases per week/month/year by scaling up the number of influenza positive SARI cases by the proportion of SARI cases tested. To estimate the proportion of ILI cases attributable to laboratory-confirmed influenza illness without population denominators require data on case counts (i.e. number of ILI cases positive for influenza virus using laboratory tests) divided by number of ILI cases from whom clinical specimens were collected for diagnostic testing multiplied by 100. To estimated number of influenza-associated ILI, we will adjust the proportion influenza-associated ILI by week, month or year multiplied by total number of ILI cases by week, month or year. ” See page 11
Prevalence rate- clarify if you are looking at point prevalence or period prevalence?	Our interest is period prevalence as stated under the type of outcome measures: ‘‘period prevalence rate is a measurement of new and preexisting of all individuals affected by the disease over a specified period of time divided by total number of people in that population. As far as prevalence estimates are concerned we will only focus on period prevalence” Page 7, type of outcomes, line 12-15
Clarify if you are interested in lab confirmed flu only? How do you deal with ILI and ICD coded studies where you are unsure of lab status?	We are interested in laboratory confirmed cases. Under the type of studies to be included, we write “We will include published estimates from studies deriving their data from sentinel surveillance systems or healthcare facilities in which human influenza infection has been verified using a valid laboratory test such as a reverse transcriptase polymerase chain reaction (RT-PCR)” Page 6, line 4-6

	Also under the discussion section, we write: "We intend to reconcile the different case definitions in different studies by matching them to ICD-9 and ICD-10 coded data "
What specific age bands do you wish to report?	Thank you. We are interested in the entire population. However, we will include studies that stratifies influenza rates in the following age groups; "0 to <2 years, 2 to <5 years, 5 to <15 years, 15 to <50 years, 50 to <65 years and over 65 years" as advised by the World Health Organization. This is found under the description of the participants, page 6.
Do you plan to run the same search strategy across the different databases?	No. Specific search strategies will be developed for each database
Reviewer 2	
While the methods provided for the systematic review of literature appear to be generally sound, the description of the anticipated methods for the meta-analysis requires further clarification. Especially, the authors should discuss the methods they will use to reconcile different case definitions, reporting thresholds, and reporting systems. The studies that are found may contain different outcome measures such as influenza-like illness (ILI), acute respiratory illness (ARI), severe acute respiratory illness (SARI), pneumonia, etc. It is not clear from the protocol, as written, how these disparate types of information will be assessed, transformed, and/or processed for combinability in the meta-analysis. Furthermore, the authors indicated they would include "seasonal influenza virus infections", but the authors have not specified the case definition that will be used. For example, the case definition might be very narrow (e.g., will only include cases having PCR laboratory confirmation) or might be very broad ("influenza-like illnesses"). Further clarification on	a) We have addressed case definition as follows "We will adopt the WHO case definitions for ILI and SARI used between 1999 and 2018. The 1999 WHO case definition of ILI was defined as "a sudden onset of fever, a temperature >38° C and a cough or sore throat in the absence of another diagnosis". In 2018, ILI is now defined as "an acute respiratory illness with measured temperature of ≥ 38° C and cough, with onset within 10 days". In 1999, SARI definition did not exist but in 2009 it was officially defined as "a sudden onset of fever > 38° C, cough or sore throat, shortness of breath or difficulty breathing, and requiring hospitalization. For those less than 5 years of age, pneumonia was used as criteria including cough or difficulty breathing. The current definition of SARI is "an acute respiratory illness with a history of fever or measured fever of ≥ 38° C and cough, with onset within the past 10 days, requiring hospitalization. We will pull studies that report laboratory confirmed influenza in patients with pneumonia that matches WHO codes (ICD-9 codes; 488.01, 488.11 and ICD-10 codes; J09.01, J09.11, J10.0). The WHO case definitions for ILI and SARI have changed many times, in 2011, 2014 and 2018 in order to facility valid comparison of disease occurrence over a period of time, and increase the sensitivity and specificity in reporting. See page 8, first paragraph. b) In terms of reporting threshold: "We will define country burden disease estimates yielded from passive sentinel surveillance data (e..g. ILI) as lower threshold and upper threshold for active sentinel surveillance data (e.g. SARI). Passive sentinel surveillance substantially yields lower estimates compared to active surveillance data" Page 11, line 3-6.

the case definition should be provided.	
The statement “Our search will not place any search limitation on the outcome of the burdens of seasonal influenza or date except for the study location.” Seems to be at odds with the statement earlier in the document, where it was indicated that the authors would be excluding data from the pandemic of 2009-2010.	Thank you. We have deleted this statement. In the protocol we emphasis exclusion of studies or data published within the pandemic period which was between April, 2009 and August, 2010. We write as follows “We will exclude all studies that use data reported between 31 January 2009 and 1 November 2010 including all studies where study duration overlaps or combines the pandemic and non pandemic- periods” See page 6 , a section on participants.
Are the outcomes clearly defined? As indicated in the response to Question 4, the outcomes should be more clearly defined. Additionally, it is unclear how the "population at risk" will be assessed for the incidence rate. This will be especially important for seasonal influenza due to potential pre-existing and cross immunity. There should be some discussion of how this will be considered as well.	We defined our outcomes as follows “If burden estimates are reported by week or month, we will calculate yearly burden estimates based on methods provided in the WHO manual for estimating disease burden associated with seasonal influenza. Where studies provide relevant data for the catchment population, we will pool burden proportions of all cases sampled among SARI cases from whom clinical specimen were tested by week/month/year by dividing the total number of SARI cases by month/week/year and multiplying it by 100%. We will adjust for true total number of influenza-associated SARI cases per week/month/year by scaling up the number of influenza positive SARI cases by the proportion of SARI cases tested. To estimate the proportion of ILI cases attributable to laboratory-confirmed influenza illness without population denominators require data on case counts (i.e. number of ILI cases positive for influenza virus using laboratory tests) divided by number of ILI cases from whom clinical specimens were collected for diagnostic testing multiplied by 100. To estimated number of influenza-associated ILI, we will adjust the proportion influenza-associated ILI by week, month or year multiplied by total number of ILI cases by week, month or year. ”
Also, the definitions of “mortality rate” and "prevalence rate" as presented is incorrect. These should both be defined as proportions, with the numerators and denominators clearly described.	Thank you for pointing out errors in our definitions. We have now incorporated denominators and our definitions are as follows: Mortality rate is defined as the number of deaths in a specific age group due to seasonal influenza, divided by population of age group expressed in 1,000 person years. Attack rate refers to number of new cases of seasonal influenza disease during specified time interval, divided by the

	number of persons or age groups at risk in the population at start of time interval. Hospitalization rate is a number of influenza inpatient admissions over a specific time and geographical area divided by the population in that age group, expressed in terms of 1000 people days. Incidence rate is the number of new cases during a specified time per population at risk in a given time period whereas period prevalence rate is a measurement of new and preexisting of all individuals affected by the disease over a specified period of time divided by total number of people in that population. Page 7, first paragraph, line 5-14 We further defined our outcomes as follows “If burden estimates are reported by week or month, we will calculate yearly burden estimates based on methods provided in the WHO manual for estimating disease burden associated with seasonal influenza. Where studies provide relevant data for the catchment population, we will pool burden proportions of all cases sampled among SARI cases from whom clinical specimen were tested by week/month/year by dividing the total number of SARI cases by month/week/year and multiplying it by 100%. We will adjust for true total number of influenza-associated SARI cases per week/month/year by scaling up the number of influenza positive SARI cases by the proportion of SARI cases tested. To estimate the proportion of ILI cases attributable to laboratory-confirmed influenza illness without population denominators require data on case counts (i.e. number of ILI cases positive for influenza virus using laboratory tests) divided by number of ILI cases from whom clinical specimens were collected for diagnostic testing multiplied by 100. To estimated number of influenza-associated ILI, we will adjust the proportion influenza-associated ILI by week, month or year multiplied by total number of ILI cases by week, month or year”
If statistics are used are they appropriate and described fully? The statement "We anticipate a wide variation in the identified results as such pooled data will be tested for heterogeneity and non-	Thank you. We have revised this statement for clarification as follows in the manuscript. “We anticipate heterogeneity in the pooled studies due to different case definitions of SARI and ILI and origin of data for the burden of disease (active and passive surveillance) thus heterogeneity will be tested using the Chi-square test and I-square test statistic. We will consider

heterogeneity by deliberately dropping one study with the most variability one at a time from the analyses." Requires further clarification, as it is not clear what this means or the intention of this analysis.	a significance level of α = 0.1 for Chi-square test and I-squared statistic of >50% to reflect significant heterogeneity. Heterogeneity and non-heterogeneity will be tested by deliberately dropping studies with high risk of bias one at a time from the analyses. If statistical heterogeneity is present, a subgroup analyses will be undertaken to examine the source of the poor data quality. Where there is significant heterogeneity, meta-analysis will not be performed. Only studies with similar risk of bias assessment will be pooled in a meta-analysis. Page 11, paragraph 2 line 3 into next page.
It is also not clear from the description of the analysis what study characteristics will be included as covariates in the meta-analysis (for example, whether the study was active or passive, the income category of the country, etc.). There should be more discussion around the anticipated covariates.	We revised the manuscript to capture the following; "If statistical heterogeneity is present, a subgroup analyses will be undertaken to examine the source of the poor data quality. We will include case definitions of influenza, passive or active studies, representation of the catchment area, age, gender, seasonality (tropical or subtropical), duration and type of study as covariates in the Meta analysis". Page 10, second paragraph line 9.
Are the study limitations discussed adequately? The biggest limitation that the authors have not appeared to address is the disparity in influenza burden reporting and publication that would lead to a high chance of non-combinability between studies. The mitigation approaches that the authors plan to take against this potential limitation should be discussed in more detail	We write in the manuscript "We anticipate several limitation in our study related to bias in influenza reporting and estimating burden of the disease. Firstly, pooled data from studies will be limited to respiratory infections such as ILI and SARI. As such there is possibility of underestimating influenza related burden caused by other clinical manifestation such as myocardial event triggered by influenza infection. Secondly, ILI surveillance data do not have a known population denominators and many people in the communities or catchment areas may not report influenza associated disease thus making it difficult to extrapolate, for example, incidence rates. Assuming enough information is provided in the studies we will adjust the estimates by using the methodology provided in a similar study. Thirdly, while we will take precautions to review studies for quality and relevance, often bias resulting from case definitions (error in coding cases), diagnostic sampling and diagnostic assays are inevitable in eligible studies thus difficult to determine precisely the disease burden estimations once we pool data for analysis. We deliberately intend to use different WHO case definitions of SARI and ILI. However, the implications with this is that much older version of case definition are highly sensitive to children under age of 5 and less sensitive to older children and adults. Further implications of the use of different case definitions is that pooled estimates may not be a reflection of true influenza burden in the population. We intend to reconcile the different case definitions in different studies by matching them to ICD-9 and ICD-10 coded data (ref supplementary file)

	to be comparable between themselves and help in harmonization and interpretation of data" Page 13 and 12 second paragraph.
Reviewer 3	
In the abstract (page 2) the authors refer to using "a Meta command". I suggest they change this to read "a meta-analysis".	Thank you. We have amended this accordingly. Page 2, Method and analysis, line 12.
In the introduction (page 4) the authors refer to only two groups, children <5 and the elderly as those most commonly affected by influenza-related complications. They could edit this to also include pregnant women and persons with chronic medical conditions.	We edited this to "Seasonal influenza affects individuals of all ages but complications are more common in those younger than 5 years of age, frail adults over 65, pregnant women and persons with chronic medical conditions" Page 4, line 8-10
On page 5, the authors state that "Seasonal influenza activity in the tropical region is not strictly seasonal as it occurs throughout the year, with bi-seasonal peaks in summer and winter". Can they be more explicit as to when exactly "summer" and "winter" are in the context of sub-Saharan Africa where they intend to conduct the study?	Thank you and we revised accordingly. "Since influenza transmission occur throughout the tropical and sub-tropical areas in Africa, we will consider studies with data reported weekly or monthly for at least a year. The peak periods are between March and September but tend to vary from year to year depending on the type and sub-type of human influenza in circulation" Page 6, line 6-9; section Type of studies to be included
On page 6, the authors state that "Studies reporting the burden in age clusters not representative of these subpopulation will be excluded." Could they state, upfront, what age clusters they plan to consider in their analysis?	Thank you. We are interested in the entire population. However, we will include studies that stratifies influenza rates in the following age groups; 0 to <2 years, 2 to <5 years, 5 to <15 years, 15 to <50 years, 50 to <65 years and over 65 years. Page 6, line 1-2
On page 7, the authors state "We will exclude economic studies that report burden indicators such as financial costs associated to influenza, quality-adjusted life years (QALYs) or disability-adjusted life years (DALYs)". Is there any particular reason for them excluding economic burden data from the review? If so, can they state it here?	We deleted this statement as it is beyond the scope of our current work

On page 7, the authors state “The titles searched by hand will be retained into the database search for a full text.” This sentence seems to be incomplete. Can they rephrase?	Thank you. We deleted this statement.
On page 8 where the authors state “a Meta command”, I suggest they state “meta-analysis to aggregate estimate measures” or better still properly refer to the “metan command” in stata if that is the intention.	We changed this to meta-analysis as suggested. See page 10, line 15
On page 10, the last sentence that reads “It can also be a predictive indicator to aid estimates of additional capacities that may be may needed to deal with a new pandemic activity in an event it occurs” is not very clear. Could the authors consider to rephrase?	We revised as follows: “Seasonal influenza burden estimates that provide baseline data can provide valuable information with which to compare annual influenza outbreaks with unusual outbreak events. This information can serve as a predictive indicator for new events such as an influenza pandemic and systematically aid pandemic planners to plan for additional capacities and resources (stockpile of antivirals and antibiotics etc) needed to deal with a severity or new pandemic activity” Page 13, line 7-11

VERSION 2 – REVIEW

REVIEWER	Nita Madhav Metabiota, USA
REVIEW RETURNED	04-Aug-2018

GENERAL COMMENTS	Thank you to the authors for such a thoughtful response to the reviewer feedback. The manuscript has had significant, positive enhancements. To finalize the protocol, the following minor adjustment should be considered: - The proposed denominator for the attack rate calculation is indicated as "the number of persons or age groups at risk in the population". It is not clear that the number of age groups at risk would be appropriate in the denominator, especially since the number of age groups could change depending on how the age bands are defined.

REVIEWER	Gideon Emukule

	CDC-Kenya
REVIEW RETURNED	20-Jul-2018

GENERAL COMMENTS	All the comments have been sufficiently addressed.

VERSION 2 – AUTHOR RESPONSE

Letter of responses to reviewer comments	
The editor and reviewer requested us to make minor changes to the manuscript before publication. We have addressed these comments below and taken the opportunity when revising this protocol to further sharpen the writing and correct occasional typographic and other errors.	
Editor comments	Responses
Please revise the 'Strengths and limitations' section of your manuscript (after the abstract). This section should contain five short bullet points, no longer than one sentence each, that relate specifically to the methods.	Thank you. We made the following changes. • This systematic review assess the epidemiological burdens of seasonal influenza without placing any restriction on language. • The search for relevant studies will include both published and unpublished to minimize the risk for publication bias. • The strength of the evidence in this review will be assessed using the GRADE approach. • A wide variation in the case definition and diagnostic of influenza may lead to inaccurate estimates of the disease burden. • The studies that will be included in this review are observational studies which are more prone to reporting biases and may overestimate the burden of the disease.
Reviewer 1:	Response
The proposed denominator for the attack rate calculation is indicated as "the number of persons or age groups at risk in the population". It is not clear that the number of age groups at risk would be appropriate in the denominator, especially since the number of age groups could change depending on how the age bands are defined.	Thank you for your comment. We will calculate attack rates as a risk of getting flu during a specified period. We revised the definition as follows; Overall attack rate refers to number of new cases of influenza during the specified time interval divided by the number of population at start of time interval. Age specific attack rates are calculated as a number of illnesses among a specified age group divided by the

	total number of persons in the specified age population who were at risk to influenza at the start of the observation period. We assume the risk period is a year. We will stratify influenza rates in the following age groups; 0 to <2 years, 2 to <5 years, 5 to <15 years, 15 to <50 years, 50 to <65 years and over 65 years.