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Appendix

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Factors associated with the risk of HIV infection among HIV-exposed  
infants in Malawi : 2013-2020

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Ng'ambi, Wingston Felix; Merzouki, Fatma Aziza; Estill, Janne Anton Markus; Orel, Erol;  
Chimbandule, Tiwonge; Nyirenda, Rose; Keiser, Olivia

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## PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Factors Associated with the Risk of HIV Infection among HIV-exposed Infants in Malawi: 2013-2020
<b>AUTHORS</b>	Ng'ambi, Wingston Merzouki, Fatma Estill, Janne Orel, Erol Chimpandule, Tiwonge Nyirenda, Rose Keiser, Olivia

### VERSION 1 – REVIEW

<b>REVIEWER</b>	Reviewer name: Ms. Kundai Moyo Institution and Country: Apex Medical Laboratories, Malawi Competing interests: None
<b>REVIEW RETURNED</b>	06-Oct-2021

<b>GENERAL COMMENTS</b>	<p>I think its an interesting study and very relevant for Malawi and other countries in the region. I am just disappointed in the researchers in using only the secondary data and even so one source instead of multiple. Could have collected direct data from registers for a couple of sites to answer or explain some of the questions arising. Of course not every study will bring answers.</p> <p>1. First, most of the literature references are more than 5years old. I would suggest add some more current literature to ensure you are referring to current literature and updates in the HIV Early Infant Diagnosis field.</p> <p>2. Second, I did not see any reference to the programmes reports/data regarding EID and outcomes. while I am aware its aggregated and not peer reviewed, but the collection has been very systematic over the years and reference in the discussion would have helped explain some of the findings and the issue of outcomes at 24months. This data is available by health facility and all periods mentioned here, though you cannot not perform the variate analyses since its aggregated.</p> <p>3. I am not sure if I understand this statement and why 20% "Only the independent variables that were statistically significant at 20% were eligible for inclusion in the multivariable model."</p>
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<b>REVIEWER</b>	Reviewer name: Dr. Peter Flom Institution and Country: Peter Flom Consulting, New York, United States Competing interests: None
<b>REVIEW RETURNED</b>	05-Oct-2021

<b>GENERAL COMMENTS</b>	<p>I confine my remarks to statistical aspects of this paper.</p> <p>First, thanks for including your own line numbers that are actual line numbers, as opposed to what BMJ provides. This makes the review process easier.</p> <p>While the general approach is fine, I do have ..... to resolve before I</p>
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	<p>can recommend publication.</p> <p>Line 139-140 This is known as bivariate screening and cannot be recommended. It leads to many errors: Standard errors are too small, p values are too low, parameter estimates are biased away from 0. For details (and proofs) see *Regression Modeling Strategies* by Frank Harrell. It's best to use substantive knowledge to build models, but, if the authors insist on an automated method, LASSO isn't bad.</p> <p>Line 141 Forward and stepwise (which are different things) have the same problems as bivariate screening. All the output is wrong.</p> <p>(However, looking at table 2 ... it doesn't seem like this affects anything, because of the large sample; no variables seem to have been dropped. Nevertheless, you could change the text).</p> <p>Congratulations on not using p values. They are essentially meaningless with such large N. (But you put them in, for some reason, in some analyses).</p> <p>Peter Flom</p>
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<b>REVIEWER</b>	<p>Reviewer name: Dr. Marina Giuliano</p> <p>Institution and Country: Istituto Superiore di Sanita (ISS), National Center for Global Health, Italy</p> <p>Competing interests: None</p>
<b>REVIEW RETURNED</b>	14-Oct-2021

<b>GENERAL COMMENTS</b>	<p>This paper addresses an important issue. However, there many clarifications needed that prevent a final judgement on acceptability. Specific comments :</p> <p><b>ABSTRACT</b></p> <p>The abstract should be modified. The main findings of the study are that the DNA test is performed within 2 months only in 57% of the children, that prevalence is higher in the Northern region, that the decreasing trend observed up to 2015, seem to have stopped and that further data are needed to say that the observed decreased trend observed in 2019 is maintained in the following years. These are the main results to be included in the results section of the abstract and in the conclusions.</p> <p>Minor comments:</p> <p>In the Results section it would be important to specify the age of the infants tested (median ?).</p> <p>Line 41. "The overall risk" should be "The overall HIV prevalence".</p> <p>Line 45. "a decreasing trend to 4.2% in 2020". I believe this statement cannot be included because of the very low numbers for 2020.</p> <p>Sentence in line 49-50 is not a conclusion based on reported results. Also, the strengthening of EID program would not reduce mother-to-child transmission.</p> <p>The key-message should be modified accordingly.</p> <p>The sections "What is known about the subject (not study)" and "What the study adds " should be modified, extended and bullet points should be used.</p> <p>For the section "What is known about this subject" I think that the focus is not EID but prevalence and risk factors for HIV pediatric infection in Malawi</p> <p>For the section "What the study adds" I would add that there is a still significant risk of acquiring HIV for children in Malawi.</p> <p><b>INTRODUCTION</b></p> <p>Line 79-80. The sentence : Globally, most of the children living with HIV are found in Africa" could be omitted (same concept in the</p>
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	<p>following sentence).</p> <p>Lines 81-82. The authors mention the Spectrum software. Maybe they should add a reference</p> <p>Lines 96-98 should be moved after first sentence of the page (line 89), since it is a general statement about the importance of EID. The two sentences should be combined and re-organized since they report the same concepts.</p> <p>Lines 89-91 and 93-96. The two sentences about WHO should be combined, possibly as follows: "The WHO guidelines recommend that all infants exposed to HIV during pregnancy, labour, delivery and breastfeeding have HIV status ascertainment by the age of 6 weeks with follow-up tests at 12 and 24 months". After this sentence the authors could start the specific part about Malawi saying that Malawi started the EID programme in 2009 and that the DNA-PCR is commonly used to test HIV in HEI registration in the early infant diagnosis programme.</p> <p>Line 102. "HEI tested EID DNA PCR" could be "DNA-PCR tested HEI"</p> <p>Line 103. Analyses are needed not only for EID program quality improvement but to assess the effectiveness of PMTCT strategies.</p> <p>Line 106. "trends" could be omitted. ..." factors associated with the risk of HIV infection..."</p> <p><b>METHODS</b></p> <p>The authors should clarify an important issue: they say that the LIMS database contains DNA-PCR data for HEI aged 24 months and below. Then at line 130-131 they say that rapid diagnostic tests are done at 12 months and 24 months. Are they using only PCR data (performed up to 24 months ?) or the data for 12 and 24 months refer to serologic tests ?</p> <p>Patient and public involvement statement</p> <p>First sentence should be re-phrased possibly as follows:          "To determine the risk of HIV infection in HIV-exposed infants data on HIV status need to be collected in the implementations of the EID programme in Malawi."</p> <p><b>RESULTS</b></p> <p>Lines 179-180. The authors say that after 2018 there was decrease in the number of HEI tested for HIV. However, the numbers for 2019 are similar to those in preceding years and for 2020 the authors considered only January-June data, so the comparison is not appropriate.</p> <p>Line 186. The subtitle should reflect that of Table 2: "Factors associated to HIV prevalence among HIV-exposed infants with HIV DNA PCR test" and should include paragraph in lines 187-192 and paragraph in lines 204-212. However, lines 189-192 should be deleted since they repeat what reported in lines 205-207 (by the way, what reported in line 189 contradict what reported in line 205). Second subtitle should be "Temporal and spatial distribution of the HIV prevalence"</p> <p>Line 195. should be deleted since the authors describe first the temporal distribution.</p> <p>Line 197-198. The authors say that that there was a decreasing trend of prevalence to 4.2% in 2020 but the numbers are too small to support the statement.</p> <p>If the authors want to include Figure 1 they should describe it better. It is OK to report that the 3 regions experienced an increase in HIV prevalence between 2015 and 2017. However, the sentence in line 200-201 does not add anything to the findings of Table 2. Looking at the Figure one could say that the prevalence seems similar in the 3 regions but in the last years (starting 2018) it seems higher in the Northern region.</p> <p>The paragraph "Spatial distribution of HIV infection" should be put together with the one of Temporal distribution and re-organized in order to avoid repetitions.</p> <p>Line 220 "risk" should be "prevalence"</p> <p><b>DISCUSSION</b></p> <p>Line 232. The authors say : "The highest risk of HIV infection was observed among the HEI tested in 2017". However, this sentence is without an explanation/comment. The authors could report the</p>
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	<p>decreasing trend observed in the first years of the analysis and the observed increase in 2017-2108.</p> <p>Line 236. "risk of HIV acquisition" should be "HIV prevalence"</p> <p>Line 238. "setting India" something is missing in the sentence</p> <p>Lines 245-247. The authors say : "Although the risk of HIV infection has been reported to be higher amongst the female than the female population (?), we observed similar risk in HIV infection by sex of the child". However, according to what reported in Table 2 this is not true.</p> <p>Lines 248-255 report the same findings of the first paragraph. The two paragraphs should be combined.</p> <p>Lines 256-264. Here the authors should report the trend over the years studied. According to what reported in Table 2, the authors cannot say that there is a decreasing trend, since the prevalence is quite stable between 2013 and 2016, then there is an increase in 2017 and 2018 and then a new decrease in 2019 (for the year 2020 no conclusion can be drawn). The authors should comment these data. Probably the main conclusion is that there is no significant decrease and much improvement is still to be achieved.</p> <p>Line 266. Is the objective of the study to improve the EID programmes ?</p> <p>Table 1.</p> <p>Age at sample draw, first line should be 0-2 (see line 176).</p> <p>Were the categories "12-17 months" and "18-24 months" tested with PCR ?</p> <p>The sample size for the Northern region is limited and this should be reported among the limitations.</p> <p>Same for samples analyzed for the year 2020</p> <p>Table 2.</p> <p>HIV prevalence in "6-11", "12-17", "18-24" months is 22.71, 41.46 and 51.72 respectively. These figures are very misleading. The sample sizes are limited and it is not clear if these are DNA PCR or serology. Are the children counted only once in the study ?</p> <p>Figure Legends are missing</p>
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## VERSION 1 – AUTHOR RESPONSE

Dear Editor for BMJ Paediatrics Open

Many thanks for reviewing our paper. We have addressed the reviewer comments point-by-point as shown below.

Kindest regards  
Wingston

### 1. Figures below 300 dpi

Please ensure that your figures are a minimum of 300 dpi and a maximum of 600 dpi. For online reviewing we do not require print quality images and the larger file sizes can slow down the running of the review process.

Please see the following link for further details on preparing images for submission:

<http://group.bmj.com/products/journals/instructions-for-authors/formatting#figures-illustrations>

Response: We have updated the figures and are now 600 dpi.

### 2. Supplementary file / Appendix

Please be informed that this should be in PDF Format.

Response: We do not have any supplementary files.

Editor in Chief Comments to Author :

Delete Key message. We do not have key messages

Response: The key message has been deleted.

What this study adds section. Delete the 1st sentence as it is Methods  
Response: The key message has been deleted.

Conclusion delete the 1st sentence. It is journal policy not to describe a study as the first. This is upto others to decide following publication. (see instructions to authors).  
Respond in full to the reviewers  
Response: The key message has been deleted.

Associate Editor

Comments to the Author:

Many thanks for your submission to BMJ Paediatrics open. Our reviewers and myself found this an insightful and important article, but still requires some work prior to publication. Please refer to our reviewers' comments and make the suggested clarifications and changes.

Response: Many thanks for the interest in this paper. We have made all the required changes as per the review.

Yours Sincerley,

Gareth Lewis

Reviewer: 1

Dr. Peter Flom, Peter Flom Consulting

**Comments to the Author**

I confine my remarks to statistical aspects of this paper.

First, thanks for including your own line numbers that are actual line numbers, as opposed to what BMJ provides. This makes the review process easier.

Response: Many thanks for this complement.

While the general approach is fine, I do have ..... to resolve before I can recommend publication.

Response: Many thanks for this complement.

Line 139-140 This is known as bivairate screening and cannot be recommended. It leads to many errors: Standard errors are too small, p values are too low, parameter estimates are biased away from 0. For details (and proofs) see \*Regression Modeling Strategies\* by Frank Harrell. It's best to use substantive knowledge to build models, but, if the authors insist on an automated method, LASSO isn't bad.

Response: Many thanks for suggesting a reference material. Considering the analysis that has been undertaken, the suggested reference material has no statistical methods for analysis of correlated data in which multiple imputation has been included as well. Therefore, we have maintained the data analysis approach which we feel better answer the said objectives.

Line 141 Forward and stepwise (which are different things) have the same problmes as bivariate screening. All the output is wrong.

Response: We have removed the backward selection method. The output is not wrong per se.

(However, looking at table 2 ... it doesn't seem like this affects anything, because of the large sample; no variables seem to have been dropped. Nevertheless, you could change the text).

Response: The variables were entered in the model using LRT so we followed the objective approach to variable selection in the model.

Congratulatoons on not using p values. They are essentially meaningless with such large N. (But you put them in, for some reason, in some analyses).

Response: Many thanks for this complement.

Peter Flom

Reviewer: 2

Ms. Kundai Moyo, Apex Medical Laboratories

**Comments to the Author**

I think its an interesting study and very relevant for Malawi and other countries in the region. I am just

disappointed in the researchers in using only the secondary data and even so one source instead of multiple. Could have collected direct data from registers for a couple of sites to answer or explain some of the questions arising. Of course, not every study will bring answers.

Response: Many thanks for this complement. We basically used HIV testing data captured in the Laboratory Management Information System. It would have indeed been great if we could have used some primary data from the registers but unfortunately the programme had just introduced a comprehensive new set of registers (HIV DNA-PCR sample log) in late 2019 and in 2020, the facilities had not yet fully transitioned to this register. Therefore, for most sites, the data was not complete in 2020.

1. First, most of the literature references are more than 5 years old. I would suggest add some more current literature to ensure you are referring to current literature and updates in the HIV Early Infant Diagnosis field.

Response: We have updated the references.

2. Second, I did not see any reference to the programmes reports/data regarding EID and outcomes. While I am aware its aggregated and not peer reviewed, but the collection has been very systematic over the years and reference in the discussion would have helped explain some of the findings and the issue of outcomes at 24 months. This data is available by health facility and all periods mentioned here, though you cannot not perform the variate analyses since its aggregated.

Response: We have added the Malawi HIV programme reports. Reference 9 "Malawi Ministry of Health, Government of Malawi Ministry of Health Integrated HIV Program Report April - June 2021, no. June, pp. 1-37, 2021."

3. I am not sure if I understand this statement and why 20% "Only the independent variables that were statistically significant at 20% were eligible for inclusion in the multivariable model."

Response: The sentence "Only the independent variables that were statistically significant at 20% were eligible for inclusion in the multivariable model." has been deleted.

Reviewer: 3

Dr. Marina Giuliano, Istituto Superiore di Sanita (ISS)

### **Comments to the Author**

This paper addresses an important issue. However, there many clarifications needed that prevent a final judgement on acceptability.

Response: Many thanks for this complement.

Specific comments :

### **ABSTRACT**

The abstract should be modified. The main findings of the study are that the DNA test is performed within 2 months only in 57% of the children, that prevalence is higher in the Northern region, that the decreasing trend observed up to 2015, seem to have stopped and that further data are needed to say that the observed decreased trend observed in 2019 is maintained in the following years.

These are the main results to be included in the results section of the abstract and in the conclusions.

Response: We have included these in the abstract and conclusion.

Minor comments:

In the Results section it would be important to specify the age of the infants tested (median ?).

Line 41. "The overall risk" should be "The overall HIV prevalence". Response: This has been changed accordingly

Line 45. "a decreasing trend to 4.2% in 2020". I believe this statement cannot be included because of the very low numbers for 2020. Response: This has been changed accordingly

Sentence in line 49-50 is not a conclusion based on reported results. Also, the strengthening of EID program would not reduce mother-to-child transmission.

The key-message should be modified accordingly. Response: This cited sentence has been deleted.

The sections "What is known about the subject (not study)" and "What the study adds " should be modified, extended and bullet points should be used.

For the section "What is known about this subject" I think that the focus is not EID but prevalence and risk factors for HIV pediatric infection in Malawi

For the section "What the study adds" I would add that there is a still significant risk of acquiring HIV for

children in Malawi.

Response: Based on the editorial comments, these sections have been removed.

## INTRODUCTION

Line 79-80. The sentence : Globally, most of the children living with HIV are found in Africa” could be omitted (same concept in the following sentence).

Response: This has been omitted.

Lines 81-82. The authors mention the Spectrum software. Maybe they should add a reference

Response: The reference number 4 has been added.

Lines 96-98 should be moved after first sentence of the page (line 89), since it is a general statement about the importance of EID. The two sentences should be combined and re-organized since they report the same concepts.

Response: This has been revised accordingly.

Lines 89-91 and 93-96. The two sentences about WHO should be combined, possibly as follows: “The WHO guidelines recommend that all infants exposed to HIV during pregnancy, labour, delivery and breastfeeding have HIV status ascertainment by the age of 6 weeks with follow-up tests at 12 and 24 months”. After this sentence the authors could start the specific part about Malawi saying that Malawi started the EID programme in 2009 and that the DNA-PCR is commonly used to test HIV in HEI registration in the early infant diagnosis programme.

Response: This has been revised accordingly.

Line 102. “HEI tested EID DNA PCR” could be “DNA-PCR tested HEI”

Response: This has been revised accordingly.

Line 103. Analyses are needed not only for EID program quality improvement but to assess the effectiveness of PMTCT strategies.

Response: This has been revised accordingly.

Line 106. “trends” could be omitted. ...” factors associated with the risk of HIV infection...”

Response: This has been revised accordingly.

## METHODS

The authors should clarify an important issue: they say that the LIMS database contains DNA-PCR data for HEI aged 24 months and below. Then at line 130-131 they say that rapid diagnostic tests are done at 12 months and 24 months. Are they using only PCR data (performed up to 24 months ?) or the data for 12 and 24 months refer to serologic tests ?

Response: In the LIMS, only data for testing done at 8 weeks are entered for the HEI. The other follow-up HIV tests are not captured. This has been included as a limitation and we have recommended that such data be captured for each HEI in order to have a complete continuum of HIV tests for HEI.

Patient and public involvement statement

First sentence should be re-phrased possibly as follows:

“To determine the risk of HIV infection in HIV-exposed infants data on HIV status need to be collected in the implementations of the EID programme in Malawi.”

Response: This has been changed accordingly

## RESULTS

Lines 179-180. The authors say that after 2018 there was decrease in the number of HEI tested for HIV. However, the numbers for 2019 are similar to those in preceding years and for 2020 the authors considered only January-June data, so the comparison is not appropriate.

Response: This has been revised and comparison has stopped at 2019.

Line 186. The subtitle should reflect that of Table 2: “Factors associated to HIV prevalence among HIV-exposed infants with HIV DNA PCR test” and should include paragraph in lines 187-192 and paragraph in lines 204-212. However, lines 189-192 should be deleted since they repeat what reported in lines 205-207 (by the way, what reported in line 189 contradict what reported in line 205).

Response: This has been changed accordingly

Second subtitle should be “Temporal and spacial distribution of the HIV prevalence”



Line 195. should be deleted since the authors describe first the temporal distribution.

Line 197-198. The authors say that that there was a decreasing trend of prevalence to 4.2% in 2020 but the numbers are too small to support the statement.

If the authors want to include Figure 1 they should describe it better. It is OK to report that the 3 regions experienced an increase in HIV prevalence between 2015 and 2017. However, the sentence in line 200-201 does not add anything to the findings of Table 2. Looking at the Figure one could say that the prevalence seems similar in the 3 regions but in the last years (starting 2018) it seems higher in the Northern region.

The paragraph "Spatial distribution of HIV infection" should be put together with the one of Temporal distribution and re-organized in order to avoid repetitions.

Line 220 "risk" should be "prevalence"

Response: This has been changed accordingly.

## DISCUSSION

Line 232. The authors say : "The highest risk of HIV infection was observed among the HEI tested in 2017". However, this sentence is without an explanation/comment. The authors could report the decreasing trend observed in the first years of the analysis and the observed increase in 2017-2018.

Response: This has been changed accordingly

Line 236. "risk of HIV acquisition" should be "HIV prevalence"

Response: This has been changed accordingly

Line 238. "setting India" something is missing in the sentence

Response: This has been changed accordingly

Lines 245-247. The authors say : "Although the risk of HIV infection has been reported to be higher amongst the female than the female population (?), we observed similar risk in HIV infection by sex of the child". However, according to what reported in Table 2 this is not true.

Response: This has been revised to reflect Table 2. Sentence changed to: "Similar to other studies showing higher HIV prevalence amongst the females than the males [13], we also observed higher prevalence of HIV by female than male children."

Lines 248-255 report the same findings of the first paragraph. The two paragraphs should be combined.

Lines 256-264. Here the authors should report the trend over the years studied. According to what reported in Table 2, the authors cannot say that there is a decreasing trend, since the prevalence is quite stable between 2013 and 2016, then there is an increase in 2017 and 2018 and then a new decrease in 2019 (for the year 2020 no conclusion can be drawn). The authors should comment these data. Probably the main conclusion is that there is no significant decrease and much improvement is still to be achieved.

Response: This has been changed accordingly

Line 266. Is the objective of the study to improve the EID programmes ?

Response: The objective is to describe trends in risk of HIV infection among Malawi's HIV-exposed infants (HEI) with DNA-PCR HIV test result from 2013 to 2020.

## Table 1.

Age at sample draw, first line should be 0-2 (see line 176).

Were the categories "12-17 months" and "18-24 months" tested with PCR ?

The sample size for the Northern region is limited and this should be reported among the limitations.

Same for samples analyzed for the year 2020

Response: The northern region of Malawi is not characterized by as many people as in other regions and the numbers that we have are not surprising. Further, the northern region doesn't have as much HIV burden as the other regions. As for the data for the year 2020, we provided the footnotes explaining the cut-offs.

## Table 2.

HIV prevalence in "6-11", "12-17", "18-24" months is 22.71, 41.46 and 51.72 respectively. These figures are very misleading. The sample sizes are limited and it is not clear if these are DNA PCR or serology. Are the children counted only once in the study ?

Response: This paper is all about DNA-PCR test. No HIV serology data are included since such data are not captured in LIMS like PCR testing data. Every child is counted only once.

Figure Legends are missing

Response: The legends are included in the figures wherever necessary like Figure 1.

### VERSION 2 – REVIEW

<b>REVIEWER</b>	Reviewer name: Dr. Peter Flom Institution and Country: Peter Flom Consulting, New York, United States Competing interests: None
<b>REVIEW RETURNED</b>	07-Dec-2021
<b>GENERAL COMMENTS</b>	The authors have addressed my concerns and I now recommend publication

### VERSION 2 – AUTHOR RESPONSE

Editor in Chief Comments to Author :

A couple of changes are still needed before we can accept your paper Page 3 lines 55-57 delete "Key message: There is need for further strengthening of the Malawi early infant diagnosis 56 program to ensure that all the HIV-exposed infants are enrolled in care by eight weeks of age 57 in order to eliminate mother-to-child transmission of HIV by 2030."

Response: Lines 55-57 have been deleted

What the study adds section.

Delete the 1st sentence "This is to our knowledge the first in-depth analysis of national routine data on HIV DNA-

64 PCR tests among HIV-exposed infants with in Malawi." as it is Methods and we do not allow authors to describe their study as the first

Response: The sentence has been deleted.

Reviewer: 1

Dr. Peter Flom, Peter Flom Consulting

#### **Comments to the Author**

The authors have addressed my concerns and I now recommend publication

Response: Many thanks for the recommendation