PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A Prospective Incidence Epidemiology Study Protocol: Conducting Active Surveillance to assess the Burden of Lyme disease (BOLD) in Primary Care Practices in Endemic Areas of Six European countries
AUTHORS	Begier, Elizabeth; Pilz, Andreas; Loew-Baselli, Alexandra; Harper, Lisa; Stark, James; Bowdery, Molly; Halsby, Kate; Dzingina, Mendwas; Bézay, Nicole; Allen, Kristen; Parslow, Ben; Gessner, Bradford

VERSION 1 – REVIEW

REVIEWER	Kulkarni, Manisha University of Ottawa Faculty of Medicine, Epidemiology & Public Health
REVIEW RETURNED	31-Jan-2023

GENERAL COMMENTS	The authors present a protocol for a prospective surveillance study to assess annual Lyme disease incidence at multiple sites in high-incidence regions of Europe. Results will be used to inform a Phase 3 vaccine efficacy study. Overall, the study objectives and methods are clearly presented and well justified, but some aspects would benefit from further clarification. Specific comments: 1. Abstract, introduction: some background information on Lyme disease would be useful before providing the aims of the study 2. Abstract, methods and analysis: this should include details on the data analysis plan 3. Introduction: the last 3 paragraphs of the introduction contain many details that would be better placed in the objectives/methods, e.g. p.3 line 27-34, line 35 and p.4 line 8 (following page and line numbers on the original submission) all relate to study objectives and could be moved to the corresponding section. 4. Objectives and endpoints: under exploratory objectives in the table, it would be useful to state some examples of risk factors of interest to the study 5. Eligibility: the exclusion criteria for controls indicates “Active Lyme Disease in last 90 days”. How is this defined (e.g. will participants be eligible for inclusion if they show resolution of symptoms for at least 90 days following treatment?). 6. Case definitions: the study requires participants to have any manifestation of LD along with laboratory confirmation. How will discordant test results be interpreted, e.g. positive serology but negative PCR and/or culture? Are the authors concerned that there may be under-detection of early cases with EM when serology is less sensitive given challenges with culture.

	7. The protocol would benefit from a conclusion section noting the study limitations.

REVIEWER	Blanchard, Laurence London School of Hygiene and Tropical Medicine Faculty of Public Health and Policy
REVIEW RETURNED	13-Feb-2023

GENERAL COMMENTS	This is an exciting study aiming to inform a much-awaited trial on LD vaccine and which can have further uses for LD surveillance practice in Europe. I am reviewing this manuscript based on my knowledge of LD surveillance systems in general, not the specific clinical and microbiological procedures. I also understand that recruitment is now over, so some of my suggestions might not be applicable. Introduction: I don't think that the sentence 'due to less developed LD surveillance in Europe…' is fair. The authors compare the USA to a continent of more than 40 countries with different practices. Also, the European Commission has adopted a case definition for Lyme neuroborreliosis. It is not as comprehensive as the USA-CDC's definition for LD but should be mentioned. You might find the following source useful: Blanchard 2022 https://doi.org/10.1186/s12889-022-13669-w . Objectives and endpoints: Both sentences miss words/are incomplete. Selection of countries and sites: Could you please detail how many sites are there per country, how were the initial 11 countries selected, and which ones were they? A study limitation worth mentioning is that all 6 countries except for Sweden are in Central Europe, which might limit generalisability to the rest of Europe. While Central Europe is known to have among the highest rates, other countries such as France and the Netherlands could have also been considered. Also, the manuscript states that 'Potential sites were not selected if annual LD incidence was less than 0.5%', and later that 'it is expected that approximately 0.5% of practice panel participants per year will be newly diagnosed with LD'. The cut-off point seems arbitrary and it reads like some participating sites will not meet it at the end. Information on equity/demographics: Stating that incidence data will be documented for people of all ages and races/ethnicities seems inclusive but the study fails to document potential inequalities beyond the traditional age and gender dimensions. A key public health objective is to reduce health inequalities, which requires documenting them. Some population subgroups might be too small to produce meaningful data but attempting to documenting differences by ethnicity/race and socioeconomic status would be helpful. Also, the general incidence rates will by analysed by age, month of diagnosis, and LD risk factor but not PTLD rates – why not? Table 13: FYI, ICD-10 code G63.0 is also used for LD in surveillance reports in Slovakia. Can you please explain the procedure or analysis for: o Controls who get a LD diagnosis during the study. Do you exclude them? o Blinding of staff for procedures that both LD patients and control receive such as surveys. o How will you communication with children, especially teenagers? Will parents always be present? o Table 4 indicates that adverse events following blood draw and biopsy will be collected for controls as well, but in the same table there is no indication that this group receives such procedures. Are you planning to test all controls for LD?

	o It isn't clear if you intend to compare healthcare service utilisation between the LD and control group (see Table 2). I think it would be useful, but this would need to be clear in Table 4 and the text. Information in Table 4 about surveys also don't match that in the text. o Page 5 line 9 states that 'a proportion of patients continue to have persistent subjective symptoms'. Aren't some of these objective, ie. inflammation/signs of arthritis that remain in articulations despite treatment? Will physical signs/symptoms also be followed in visits 4-5, and if so shouldn't photographs be taken? (they are currently only specified for visit 1) o Table 4: It would be helpful to indicate in a heading that visits 4-5 are for patients with PTLD only, and under the section 'study visits' that patients with LD will be seen between 3-5 times, and controls twice (it currently reads as if it can range between 1-5 visits). LD Case definition: The reference to EUCALB's definition is not right and their definition is from 1996. Also, why are simple (non-disseminated) cases of erythema migrans not considered? It is quite standard in most case definitions and without it the study is likely to miss most cases of LD. If you don't, this study should clearly state that it focuses on late/disseminated LD only, including in the title, abstract,background, aim, objectives and methods. Lastly, I'm just noting that Lyme carditis is the only condition that relies on a single diagnostic procedure. Debates about LD and PTLD: I am sure that you are aware that Lyme disease is a controversial condition. Whether we want it or not, this impacts several aspects of the study. a) First, the literature shows that LD diagnosis and treatment is influenced by doctors' beliefs and attitudes towards Lyme disease (not only the chronic/PTLD form), eg Hinds 2019 https://doi.org/10.1177/1049732319846170; peer-reviewed report by Brunton 2017 https://eppi.ioe.ac.uk/cms/Portals/0/PDF%20reviews%20and%20summaries/Lyme b) %20disease%20stakeholder%20experiences%202017%20Brunton.pdf? c) ver=2018-04-23-155309-640). Could you please expand how you have ensured (or made your best) to train/sensitize clinics' staff to consider Lyme disease when seeing patients? This is also a study limitation: it relies on doctors' willingness and interest in Lyme disease. b) Second, the study aims to measure post-treatment Lyme disease, which is much needed. Yet, this condition is at the core of the debate. I think that this should be mentioned, for justifying both the need for measuring such condition and the definition used. I don't think that ignoring it helps. Furthermore, the study uses ISDA's definition from 2006, which is quite old and conservative, so a rationale would be useful, and its limitations mentioned. Dissemination: This study will produce standardised epidemiological data for 6 European countries – something that is currently difficult to obtain due to the heterogeneity in the systems. Will this data or at least part of it be publicly available? This would be useful for public health practice and policy.

VERSION 1 – AUTHOR RESPONSE

REVIEWER 1:

Comment 1: Abstract, introduction: some background information on Lyme disease would be useful before providing the aims of the study.

Response 1: We agree with the reviewer comment and have added background information on Lyme disease to the ‘Abstract: Introduction’ section of this manuscript.

Comment 2: Abstract, methods and analysis: this should include details on the data analysis plan

Response 2: We agree with the reviewer comment and have updated the ‘Abstract: Methods and Analysis’ section to include some details on the data analysis plan.

Comment 3: Introduction: the last 3 paragraphs of the introduction contain many details that would be better placed in the objectives/methods, e.g. p.3 line 27-34, line 35 and p.4 line 8 (following page and line numbers on the original submission) all relate to study objectives and could be moved to the corresponding section.

Response 3: We agree with the reviewer comment and have removed these details from the Introduction. Much of the information contained here was already present in the original manuscript, either in the ‘Objectives and Endpoints’ or ‘Methods and Analysis’ section. However, where not already incorporated we have added details to these sections.

Comment 4: Objectives and endpoints: under exploratory objectives in the table, it would be useful to state some examples of risk factors of interest to the study

Response 4: We agree with the reviewer comment and have now included several examples of LD risk factors of interest to the study, under Table 1 Exploratory Objectives.

Comment 5: Eligibility: the exclusion criteria for controls indicates “Active Lyme Disease in last 90 days”. How is this defined (e.g. will participants be eligible for inclusion if they show resolution of symptoms for at least 90 days following treatment?).

Response 5: In response to the reviewer’s question, participants will be eligible for inclusion if they show resolution of symptoms for at least 90 days following treatment. Therefore, the exclusion criterion ‘Active Lyme disease in last 90 days’ refers to the presence of any LD associated symptoms. The ‘Eligibility’ section has been updated to reflect this information.

Comment 6: Case definitions: the study requires participants to have any manifestation of LD along with laboratory confirmation. How will discordant test results be interpreted, e.g. positive serology but negative PCR and/or culture? Are the authors concerned that there may be under-detection of early cases with EM when serology is less sensitive given challenges with culture.

Response 6: We would like to thank the reviewer for their comment and for noticing this section of the manuscript that required further clarification. To confirm, in this study suspected LD cases will be assigned a final diagnosis based on clinical assessment and LD clinical manifestations will be recorded in line with the categories described in Table 5. A subset of enrolled cases with a final clinical diagnosis of LD will be further delineated into clinically diagnosed laboratory-confirmed LD cases through at least one study sample. EM cases can be diagnosed on the basis of clinical symptoms alone. Based on this information, we have adapted the text in the ‘LD Surveillance Case Definitions’. Regarding the reviewer’s comment on the interpretation of discordant test results, we refer the reviewer to ‘Table 5 LD Case Definitions’, which allow for a LD case to be counted as laboratory-confirmed if just one of the diagnostic lab test results is positive.

Comment 7: The protocol would benefit from a conclusion section noting the study limitations.

Response 7: We thank the reviewer for their comment. As the journal guidance for writing this protocol manuscript did not include a conclusion section, we have decided not to add one. However, to address this comment we have revised the text within the 'Strengths and Limitations' section.

REVIEWER 2:

Comment 1: I don't think that the sentence 'due to less developed LD surveillance in Europe…' is fair. The authors compare the USA to a continent of more than 40 countries with different practices. Also, the European Commission has adopted a case definition for Lyme neuroborreliosis. It is not as comprehensive as the USA-CDC's definition for LD but should be mentioned.

Response 1: We agree with the reviewer comment and have adapted the manuscript text to emphasize the heterogeneity of Lyme disease surveillance systems across Europe. Further, we have noted the European Commission's 2018 update, the adoption of a standardized case definition for Lyme neuroborreliosis in the EU.

Comment 2: Objectives and endpoints: Both sentences miss words/are incomplete.

Response 2: We thank the reviewer for their comments and have updated these sentences accordingly.

Comment 3: Selection of Countries and Sites. Could you please detail how many sites are there per country, how were the initial 11 countries selected, and which ones were they? A study limitation worth mentioning is that all 6 countries except for Sweden are in Central Europe, which might limit generalizability to the rest of Europe. While Central Europe is known to have among the highest rates, other countries such as France and the Netherlands could have also been considered.

Response 3: In response to the reviewer's comments, we have updated the 'Methods and Analysis: Site Selection' section to include details of how many active sites there are per country and how the initial 11 countries were selected. These 11 countries were as follows: Germany, Czech Republic, Sweden, Poland, Slovakia, Slovenia, Scotland (UK), Russia, Finland, Denmark, and Italy. Whilst we agree with the reviewer that additional active study sites outside of Central Europe could have been considered, the primary objective of this study is not to provide incidence estimates for Europe, but rather to vet and assess the annual LD incidence at potential Phase 3 efficacy trial sites for the VLA15 vaccine.

Comment 4: The manuscript states 'Potential sites were not selected if annual LD incidence was less than 0.5%', and later that 'it is expected that approximately 0.5% of practice panel participants per year will be newly diagnosed with LD'. The cut-off point seems arbitrary, and it reads like some participating sites will not meet it at the end.

Response 4: The cut-off point of 0.5% annual incidence was selected based on sample size requirements for the Phase 3 vaccine efficacy trial. Based on this, we have updated the 'Methods and Analysis: Site Selection' section to include this information. The reviewer is correct in their statement that some of the study sites may not meet the 0.5% cut-off (due to LD variations between years).

Comment 5: Information on equity/demographics: Stating that incidence data will be documented for people of all ages and races/ethnicities seems inclusive but the study fails to document potential inequalities beyond the traditional age and gender dimensions. A key public health objective is to reduce health inequalities, which requires documenting them. Some population subgroups might be

too small to produce meaningful data but attempting to documenting differences by ethnicity/race and socioeconomic status would be helpful. Also, the general incidence rates will be analyzed by age, month of diagnosis, and LD risk factor but not PTLD rates – why not?

Response 5: We agree with the reviewer that population subgroups will be too small, specifically non-caucasian subgroups in Europe. In the CSR, descriptive data will be presented on ethnicity of LD cases and controls. We do not plan to conduct subgroup analyses by race/ethnicity due to sample size limitations, and we have added this information to the 'Data Analysis - Analysis of Endpoints' section of the manuscript. Unfortunately, socioeconomic status of participants was not collected, and therefore we cannot analyze based on this variable. Further, we agree with the reviewer that analyzing incidence rates by PTLD rates would be a worthwhile addition to the analysis and we will discuss with our statistician to include. However, as it is currently not included in our protocol, we have not updated the manuscript.

Comment 6: Table 13: FYI, ICD-10 code G63.0 is also used for LD in surveillance reports in Slovakia.

Response 6: We agree with the reviewer comment and have added a footnote to Table 3 to explain that medical records were searched using diagnoses/key words from Table 3 as well as any diagnoses/terms that are used for LD locally.

Comment 7: Can you please explain the procedure or analysis for: Controls who get a LD diagnosis during the study. Do you exclude them?

Response 7: For Lyme disease cases who then became controls, we did have an exclusion criterion (active Lyme disease in the last 90 days). For controls who become Lyme disease cases, we ensure that their symptoms of disease start after being enrolled as a control. We have included text in the 'Eligibility' section of the manuscript based on this. We see no reason to exclude them as controls if they did not have active Lyme disease at that time. We sought to enroll the controls as soon as feasible following case identification to make them as comparable to the controls as feasible and thus it is possible they would develop Lyme disease at some later date.

Comment 8: Can you please explain the procedure or analysis for: Blinding of staff for procedures that both LD patients and control receive, such as surveys.

Response 8: There is no blinding of study site staff in this study. Surveys were completed by subjects themselves or assisted by site personnel, and the method of completion was documented. Clarifying text has been added to the 'Study Specific Procedures' section.

Comment 9: Can you please explain the procedure or analysis for: How will you communication with children, especially teenagers? Will parents always be present?

Response 9: In this study we were compliant with the different country and local requirements, and where applicable utilized age-appropriate informed consent and assent documents. Specifically, this study was set up with Adult ICDs, as well as an assent documents for younger children (6 to 10 years of age) and older children (11 years of age up to the legal age of adulthood in each country). These pediatric assent documents went together with the Guardian ICDs, where the parent/guardian of the minor also provided their consent to participate in the study on the child's behalf. The legal age of adulthood differs between countries, usually 18 (or around this age). Before this age, communication is required to be provided to both parent/guardian as well as to the participant (minor age) in an age-appropriate manner. We have updated the 'Eligibility' section of the manuscript accordingly.

Comment 10: Table 4 indicates that adverse events following blood draw and biopsy will be collected for controls as well, but in the same table there is no indication that this group receives such procedures. Are you planning to test all controls for LD?

Response 10: Adverse events (AEs) in relation to study procedures apply for LD cases only. Controls are not tested for LD and do not have samples taken in this study, and as such do not undergo study procedures such as blood draw and skin punch biopsies. Table 4 has been updated accordingly.

Comment 11: It isn't clear if you intend to compare healthcare service utilization between the LD and control group (see Table 2). I think it would be useful, but this would need to be clear in Table 4 and the text. Information in Table 4 about surveys also don't match that in the text.

Response 11: In this study, we are comparing the healthcare resource utilization of Lyme disease cases with and without persisting symptoms (including PTLD) but are not collecting healthcare resource utilization data for controls. The HCRU data collected for LD cases is not all cause but rather those that are specifically related to the LD event for which the subject is enrolled in the study. We have reviewed and updated the text in the 'Study Specific Procedures' section for LD cases and controls to ensure consistency with Table 4.

Comment 12: Page 5 line 9 states that 'a proportion of patients continue to have persistent subjective symptoms'. Aren't some of these objective, ie. inflammation/signs of arthritis that remain in articulations despite treatment? Will physical signs/symptoms also be followed in visits 4-5, and if so shouldn't photographs be taken? (they are currently only specified for visit 1).
Response 12: We agree with the reviewer's comment and have updated this sentence to 'a proportion of patients continue to have persistent symptoms'. All signs and symptoms of disease (including PTLD) will be followed at visits 4-5 but photographs are taken at Visit 1 only.

Comment 13: Table 4: It would be helpful to indicate in a heading that visits 4-5 are for patients with PTLD only, and under the section 'study visits' that patients with LD will be seen between 3-5 times, and controls twice (it currently reads as if it can range between 1-5 visits).

Response 13: We agree with the reviewer's comment and have updated the 'Study Visits' section of the manuscript to include this information for clarity.

Comment 14: The reference to EUCALB's definition is not right and their definition is from 1996. Also, why are simple (non-disseminated) cases of erythema migrans not considered? It is quite standard in most case definitions and without it the study is likely to miss most cases of LD. If you don't, this study should clearly state that it focuses on late/disseminated LD only, including in the title, abstract, background, aim, objectives and methods. Lastly, I'm just noting that Lyme carditis is the only condition that relies on a single diagnostic procedure.

Response 14: We agree with the reviewer and would like to thank them for noticing this section in the manuscript which required further clarification. We have updated the 'Data Analysis: LD Surveillance Case Definitions' section to emphasize that the definitions used in this study have been derived from clinical case definitions originally developed by EUCALB in 1996, which were subsequently updated by EUCALB Advisory Board members in 2011. To confirm, in this study suspected LD cases will be assigned a final diagnosis based on clinical assessment and LD clinical manifestations will be recorded in line with the categories described in Table 5. A subset of enrolled cases with a final clinical diagnosis of LD will be further delineated into clinically diagnosed laboratory-confirmed LD cases through at least one study sample. EM cases can be diagnosed on the basis of clinical symptoms alone. Based on this information, we have adapted the text in the 'LD Surveillance Case Definitions' section.

Comment 15: Debates about LD and PTLD: I am sure that you are aware that Lyme disease is a controversial condition. Whether we want it or not, this impacts several aspects of the study.

Response 15: We agree with the reviewer's statement and is the reason for adding PTLD assessment to the study.

Comment 16: The literature shows that LD diagnosis and treatment is influenced by doctors' beliefs and attitudes towards Lyme disease (not only the chronic/PTLD form). Could you please expand how you have ensured (or made your best) to train/sensitize clinics' staff to consider Lyme disease when seeing patients? This is also a study limitation: it relies on doctors' willingness and interest in Lyme disease.

Response 16: We thank the reviewer for their comment and have now updated the 'Methods and Analysis: Active Surveillance' section. Study specific standardized training was provided to all site staff at the beginning of the study during the Investigator Meeting, through available online training resources throughout study conduct, and via dedicated onsite visits by trained CRAs.

Comment 17: The study aims to measure post-treatment Lyme disease, which is much needed. Yet, this condition is at the core of the debate. I think that this should be mentioned, for justifying both the need for measuring such condition and the definition used. I don't think that ignoring it helps. Furthermore, the study uses IDSA's definition from 2006, which is quite old and conservative, so a rationale would be useful, and its limitations mentioned.

Response 17: We thank the reviewer for their comment and refer them to the last paragraph of the Introduction which details our rationale and justification for including an assessment of PTLD. This rationale is on the basis that A) the existing PTLD literature is heterogenous; B) PTLD is poorly characterized in terms of the size of the patient group, severity of symptoms, duration of symptoms, impact on quality of life, and health care utilization; and C) PTLD measurement will inform future cost-effectiveness analysis. We have added some additional text to this section, acknowledging the debate around PTLD.
IDSA developed the PTLD definition in 2006 as a method to stimulate standardized research. Subsequently in 2020 as an update to the treatment guidelines, they removed the PTLD research definition. To our knowledge there are no other formal definitions, and most researchers continue to use this definition. Others develop their own mechanism to define PTLD based on the data source.

Comment 18: Dissemination: This study will produce standardized epidemiological data for 6 European countries – something that is currently difficult to obtain due to the heterogeneity in the systems. Will this data or at least part of it be publicly available? This would be useful for public health practice and policy.

Response 18: As noted in the 'Ethics and Dissemination' section, we aim to publish results from this study in peer-reviewed international journals and present them at relevant conferences. Further, several posters and abstracts related to this study have already been presented at conferences. Please find additional details and examples below:

ECCMID 2022: Burden of Lyme disease (BOLD) study – A prospective Active Surveillance Study in European Endemic Regions: Interim Results

ICLB 2022: Manifestations of clinically-diagnosed Lyme borreliosis from active surveillance at 14 general practices in endemic areas in 6 European countries.

ISTTBD 2023: Preliminary Results from the Burden of Lyme disease (BOLD) Study. A Prospective Active Surveillance Study at Primary Care Practices in Endemic Regions of six European Countries.

COMMENTS RECEIVED FROM BMJ OPEN EDITORIAL OFFICE ON 02-JUN-2023:

Comment 1: Author Contributions. Please provide a more detailed contributorship statement. It needs to mention all the names/initials of authors along with their specific contribution/participation for the article. This should be stating how each author contributed to the article. It should discuss the planning, conduct and reporting of the work
in your paper. You may also consider the conception and design, acquisition of data or analysis and interpretation of data, etc.

Response 1:
We thank BMJ Open for their comment and have now expanded our Authors' Contributions statement. This includes details of who the original protocol was written by and who has amended it. Further, the initials of all co-authors who critically reviewed this protocol manuscript have been included.

Comment 2: Figures and Tables. Please limit the combined number of figures and tables to 5 in total as outlined on the Information for Authors. The rest can be uploaded as supplemental files or online data. If uploaded as a supplemental file, please make sure that it is cited in the main text as "Supplemental Figure" or "Supplemental Table." If you are unable to amend the submission to fit within the limits, please include an explanation why this is not possible in the cover letter.

Response 2: We thank BMJ Open for their comment and have made the necessary updates to our Figures and Tables accordingly. One Figure (Active Surveillance Process within GP practice panels) and one Table (Lyme Disease and related diagnoses with relevant International Classification of Diseases [ICD; procedure codes used in medical billing] codes if available) have been moved to 'Supplemental Material'. Additionally, we have combined two tables into one within the manuscript itself.

We would again like to thank the reviewers for their comments. We feel that the changes requested have greatly improved our manuscript and we look forward to your continued consideration of our work.