Transmission pathways of Shiga toxin-producing *Escherichia coli* resulting in Haemolytic Uraemic Syndrome: A Systematic Review

**Review team:** Rebecca Inman, Erica Kintz, Paul Hunter, Lee Hooper

**Background**

Shiga toxin-producing *Escherichia coli* (STEC), also known as verocytotoxin producing *E. coli* (VTEC), are gastrointestinal pathogens that cause a range of conditions including bloody or non-bloody diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome (HUS) in humans (Siegler 2003; Thorpe 2004; Walker et al. 2012). HUS is a serious and life-threatening condition, which is characterised by acute renal failure, low platelet count, and haemolytic anaemia, often following prodromal diarrhoea. Children under 5 years and the elderly are particularly vulnerable to both STEC infections and the development of HUS, where total incidences of HUS vary from approximately 3-7% in sporadic STEC cases to up to 20% in outbreaks (Corrigan and Boineau, 2001; Noris and Remuzzi 2005). Studies of STEC have suggested a number of diverse transmission pathways; including food (particularly undercooked beef, raw vegetables and unpasteurised dairy products), contaminated water, environmental (contact with farm animals, manure etc.) and person-person contact (Bardasi et al., 2015; Erickson and Doyle 2007; Ferens and Hovde 2011; Hussein and Bollinger 2005; Persad and LeJeune 2014). A separate systematic review is being carried out to address STEC transmission pathways, not specifically relating to HUS, in sporadic and outbreak cases (Kintz et al. 2015).

It is thought that the majority of STEC infections are caused by the O157:H7 serotype; although there have been an increasing number of reports of non-O157 STECs including O104, O111, O45 and O26 serotypes (Hughes et al. 2006; Noris and Remuzzi, 2005). O104:H4 was responsible for a large outbreak in Germany in 2011; which resulted in almost 4000 total STEC infections, over 800 cases of HUS and approximately 54 deaths (Frank et al. 2011; RKI.de report). Although the outbreak was mostly localised to Germany, a number of cases were reported in other countries including France, Sweden, Denmark, the USA, Canada and the UK. These cases were associated with patients having travelled to Germany or France, and presented the O104:H4 serotype indistinguishable from the serotype identified in Germany (Scavia et al. 2011). It was eventually discovered that imported fenugreek seeds were the likely source of the outbreak; although it was not possible to identify how and when the seeds became contaminated (Buchholz et al., 2011). This not only highlights the importance of identifying the source of the infection, but also identifying the process of contamination so methods of prevention can be properly implemented. The high proportion of HUS cases in this outbreak as well as the large number of deaths signifies the threat that STEC causes to worldwide public health, and the need for further investigations into identifying the sources and transmission pathways of STEC that are responsible for causing cases of HUS.

Although O104:H4 is considered to be a relatively uncommon serotype of STEC, it was responsible for the largest outbreak of STEC ever recorded and proved to be highly pathogenic and virulent (Muniesa et al. 2012). This clearly indicates the need for additional research and characterisation of non-O157 strains to assess their contribution to the disease burden. In general, virulent bacteria are distinguishable from non-virulent bacteria by the presence of...
gene-encoded virulence factors; which can be part of one or more pathogenicity islands. STEC pathogenicity has been linked to several plasmid-encoded virulence factors; including Shiga-like toxin 1 (Stx1), Shiga-like toxin 2 (Stx2), intimin (aea) and enterohaemolysin (ehxA) (Boerlin et al. 1999; Etcheverría and Padola 2013). Several studies have also identified an association between the presence of Stx2 subtypes and the development of HUS (Bonnet et al. 1998; Brandal et al. 2015; Ethelberg et al., 2004; Fernández-Brando et al. 2011; Schimmer et al. 2008) We will therefore investigate these findings to determine whether there is a plausible link between the presence of certain virulence factors and the development of HUS from STEC infections.

Objectives

Primary objective:

To systematically review the routes of transmission of Shiga-toxin producing Escherichia coli (STEC) that cause haemolytic uremic syndrome (HUS).

Secondary objectives:

We will also assess the following questions in order to evaluate the transmission and impact of STEC-related HUS in a public health context:

1. Which strains/serotypes of STEC are associated with the development of HUS?
2. Are there particular virulence factors associated with STEC-related HUS?
3. What proportion of STEC cases result in HUS?
4. What are the rates of secondary (person-to-person) transmission of STEC-HUS?
5. What are the risk factors for developing STEC-HUS in young children and the elderly?
6. What are the long term health impacts of STEC-HUS patients?

Criteria for inclusion

Types of study:

We will consider epidemiological studies including case-control, prospective and retrospective cohort studies.

Participants:

We will consider all studies that involve a minimum of one person with HUS that has resulted directly from STEC infection, which can be sporadic or part of an outbreak. There will be no limitations of the number of STEC cases. Patients must be diagnosed with STEC and HUS using the pre-defined criteria (see outcomes).

Participants will not be restricted by age, gender or any other limiting factor.

Comparators:

For case-control studies, risk factors for STEC and/or HUS can be compared to controls that either have an unrelated illness (non-gastrointestinal) or otherwise healthy individuals.

Exposures:
All potential exposures including food, air, water, environmental, human and animal will be included in this review. In most cases this will be identified through a questionnaire which will have been completed by the patient after exposure.

Studies that only consider secondary (person-to-person) transmission of STEC will be considered if they fit all of the inclusion criteria. A primary source of STEC does not necessarily need to be identified as secondary transmission is considered a transmission pathway in itself.

**Outcomes:**

*Primary outcome(s):*
- STEC infections that cause HUS in at least one person

*Secondary outcome(s):*
- Strains of STEC causing HUS
- Virulence factors of STEC associated with the development HUS
- Identify risk factors for exposures to STEC and/or risk of acquiring HUS from STEC infections

Criteria for identification of STEC:
- Identification of virulence factors Shiga-like toxin (Stx) 1 or 2, attaching and effacing factors or free verocytotoxin  
  OR  
- Identification of STEC through culture such as sorbitol agar (*E. coli* O157:H7 does not ferment sorbitol, distinguish from other faecal *E. coli*)  
  OR  
- Detection of antibodies in the blood (e.g. antibodies to O157 or shiga toxin)

Criteria for diagnosis of STEC-related HUS:
Patients with STEC infection diagnosed by previously stated criteria and one or more of the following symptoms:
- Acute renal failure
- Haemolytic anaemia
- Low platelet count (thrombocytopenia)

We will only consider studies that have STEC-related HUS cases with a defined time period between STEC diagnosis/onset of symptoms and sample collection, as this will determine which method is a valid measure of STEC diagnosis. Studies should also include the risk factors for acquiring STEC infections with (where appropriate) statistical analysis.

**Search strategy**

Studies will be identified by a search strategy from the following electronic databases:
- Medline (via OVID)
- Embase (via OVID)
- Web of Science
The following will also be searched for grey literature:

- Scopus
- http://opengrey.eu
- http://cdc.gov
- http://who.int
- http://ethos.bl.uk

The search will not be restricted by language or publication status. All studies contained in previous relevant systematic reviews will be screened for inclusion.

Bibliographies of included studies will be checked for other relevant studies. Searches will not be limited by language or time period.

The search strategy developed for Medline will combine text and indexing terms, truncation, adjacency searching and Boolean operators. The strategy will be tested against a set of five studies that have pre-selected to fit the inclusion criteria in order to ensure that the strategy is picking up the relevant studies (Byrne et al. 2015; Friesema et al. 2014; Frank et al. 2011; Mody et al. 2012; Vaillant et al. 2009). The search strategy for Medline outlined in appendix 1 has succeeded in identifying all these papers and will be adapted for use in other electronic databases.

**Methods**

**Data collection:**

Screening of titles and abstracts against the inclusion criteria will be carried out independently by two reviewers, and full text versions of identified studies will be collected for assessment. Inclusion/exclusion will be assessed using a standardised form (see appendix 2). Differences in opinion will be resolved with discussion and, where necessary, arbitrated by a third reviewer.

The draft data extraction form (appendix 3) will be tested on the first two studies that fulfil the inclusion criteria to ensure that the form captures all the data that is needed from these studies. The data from all included studies will be extracted in duplicate using the standardised form, any differences will be resolved with discussion or arbitrated by a third reviewer. We will also attempt to contact the authors/researchers of the study to clarify or obtain missing data as required.

**Risk of bias assessment:**

The risk of bias of each of the included studies will be assessed independently by two reviewers using a modified version of the Newcastle-Ottowa scale for case-control and cohort studies (see appendix 4). As well as the generic criteria, we will also assess the methodological quality of the case diagnosis and the outcome measures such as risk factors and control measures for confounding variables. Choices that indicate a LOW risk of bias will be followed with a star (*), and risk of bias will be rated either HIGH or MODERATE or LOW based on the number of stars that they have been awarded (maximum of 6 stars for case-control and cohort studies).

*Case-control and cohort studies*

≥ 5 stars will indicate a study with LOW risk of bias
3-4 stars will indicate a MODERATE risk of bias

<3 stars will indicate a HIGH risk of bias

Risk of bias assessments will question the method of selecting controls, whether or not all potential exposures were considered, how the exposure was ascertained, whether there were reasons given for excluding patients or data and whether or not there was follow up of participants to assess long term health impacts (e.g. chronic kidney failure, complete recovery, mortality).

A summary table of the outcomes of the quality assessment will be available as a supplementary addition in the final review.

**Data synthesis:**

Results of the searches and the process of including studies will be presented in the PRISMA 2009 flowchart (see appendix 5). A summary table will be used to present the findings from the included studies as well their corresponding quality assessment ratings determined by the Newcastle-Ottowa scale. A narrative summary of the characteristics and quality of each study will also be included in the main review.

Data regarding identified sources of STEC and overall incidences of HUS will be combined in order to assess risk factors for the development of STEC-related HUS. Depending on availability of appropriate data, these will be pooled into a meta-analysis using Review Manager (RevMan) software, and heterogeneity will be quantified using $I^2$. Publication bias will be assessed alongside the meta-analysis and presented as a funnel plot.

A similar data synthesis strategy will be carried out separately for the secondary objectives. However, if there is insufficient data available then a meta-analysis will not be possible, and the outcomes will instead be discussed narratively with indications of a need for further reviews to be carried out.

**Dissemination strategy:**

We plan to publish this systematic review in a peer reviewed journal and the findings will also be presented at future academic conferences. The ongoing progress of this review will be presented at the Health Protection Research Unit for Gastrointestinal Infections (HPRU-GI) annual conference in March 2016.

**References:**


characterization of isolates and identification of risk factors for haemolytic uremic syndrome. *BMC Infectious Diseases*, 15(1).


Rki.de, (2011). Final presentation and evaluation of epidemiological findings in the EHEC O104:H4 Outbreak Germany 2011. [online] Available at:
Appendix 1: Search terms and strategy for MEDLINE (Ovid)

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to Present

Search 1 – *Escherichia coli*

1. Exp Escherichia coli
2. exp shiga-toxigenic Escherichia coli
3. E* coli. ti,ab.
4. (shiga adj3 toxi*). ti,ab.
5. STEC. ti,ab.
7. Verotoxi*. ti,ab.
8. VTEC. ti,ab.
9. (Enteroh*emorrhagic adj3 E* coli). ti,ab.
10. EHEC. ti,ab
12. (Stx1 or Stx2). tw.
13. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12

Search 2 – *Haemolytic uraemic syndrome*

14. Exp Hemolytic-uremic syndrome
15. (He*molytic adj3 ur*emic). ti,ab.
16. HUS. ti,ab.
17. 14 OR 15 OR 16

Search 3 – Combining search results

18. (animals not (humans and animals)).sh
19. 13 AND 17
20. 19 NOT 18
Appendix 2: Study eligibility screening form

<table>
<thead>
<tr>
<th>Essential inclusion criteria</th>
<th>Yes / No / Unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Does the study include both STEC and HUS cases?</td>
<td>Yes / No / Unclear</td>
</tr>
<tr>
<td>2. Is the study a case-control or cohort study?</td>
<td>Yes / No / Unclear</td>
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<tr>
<td>3. Has the study assessed the transmission pathways for STEC and STEC-related HUS cases?</td>
<td>Yes / No / Unclear</td>
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<td>4. Has the study linked STEC as the cause of HUS?</td>
<td>Yes / No / Unclear</td>
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<tr>
<td>5. Has the study adequately diagnosed STEC with one or more of the following methods:</td>
<td>Yes / No / Unclear</td>
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<tr>
<td>- Identification of virulence factors Stx 1, Stx 2 or free veroctotoxin using PCR</td>
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<td>- Detection of antibodies to either O157 or shiga toxin in the patients’ serum</td>
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<td>- Identification of STEC through sample cultures (either directly or obtained information from laboratories)</td>
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<tr>
<td>6. Has the study adequately diagnosed HUS with one or more of the following symptoms:</td>
<td>Yes / No / Unclear</td>
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<tr>
<td>- Acute renal failure</td>
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<tr>
<td>- Haemolytic anaemia</td>
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<td>- Low platelet count (thrombocytopenia)</td>
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</table>

Note: To be included studies must answer “yes” to all of the above questions

If any answers are “uncertain” please state any further action that should be taken or any questions that need answering in order for a final decision to be made

Circle final decision:

Include / Exclude / Uncertain

Further action to be taken/ questions for clarification:

Reasons for exclusion:
### Appendix 3: Data extraction form

<table>
<thead>
<tr>
<th>1. General information</th>
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<tbody>
<tr>
<td><strong>Name of reviewer</strong></td>
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<td><strong>Date of extraction</strong></td>
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<td><strong>Publication type (e.g. journal article, thesis, letter)</strong></td>
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<td><strong>Country of origin/language</strong></td>
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<td><strong>Method of language translation (if applicable)</strong></td>
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<tr>
<th>2. Bibliographical information</th>
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<tr>
<td><strong>Study title</strong></td>
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<tr>
<td><strong>Primary author name (surname, initial)</strong></td>
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<tr>
<td><strong>Other author names (surname, initial)</strong></td>
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<td><strong>Journal/source reference (journal name, volume, pages)</strong></td>
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<td><strong>Year published</strong></td>
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<td><strong>Is this study part of a series of other published papers? If so give details:</strong></td>
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<th>3. Study information</th>
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<tr>
<td><strong>Type of study/study design</strong></td>
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<td><strong>Primary aim(s) of study</strong></td>
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<td><strong>Secondary aims(s) of study</strong></td>
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<td><strong>Study duration (days/months/years)</strong></td>
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<td><strong>Type of STEC case (sporadic/outbreak)</strong></td>
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<td>Total number of STEC cases</td>
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<td>Total number of HUS cases</td>
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<td>Total number of controls</td>
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<tr>
<th>Age of case (STEC and HUS) participants (range/median/mean)</th>
<th>Mean &amp; SD or median &amp; IQ range</th>
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<th>Age of control participants (range/median/mean)</th>
<th>Mean &amp; SD or median and IQ range</th>
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4. Methods

Method(s) of identifying STEC

HUS diagnosis criteria

Patient data collection method(s) (e.g. questionnaire, interview)

Time between infection and data collection (e.g. interview, questionnaire completion)

Method(s) of analysis (e.g. statistical tests, presentation of data)

Were there patient follow up reports? If so give details:
### 5. Results and discussions

<table>
<thead>
<tr>
<th>Strain(s)/serotype(s) of STEC identified</th>
<th>Virulence factors identified</th>
<th>Univariate or Multivariate analysis?</th>
<th>What were the adjusting factors/confounding variables? (e.g. geographical location, level of urbanisation, age, gender, travel, season or time of year)</th>
<th>STEC sources/risk factors investigated</th>
<th>Numerical results (p-values, OR, RR)</th>
<th>Descriptive results</th>
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<tr>
<th>STEC sources/risk factors identified</th>
<th>Type of data (e.g. OR, RR)</th>
<th>Numerical relationship (including 95% CI or p-value)</th>
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<tbody>
<tr>
<td>1. Consumption of raw/undercooked meat</td>
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<td>2. Consumption of unpasteurised dairy products</td>
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<td>3. Consumption of other contaminated food products</td>
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<td>4. Contact with animals/manure</td>
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<td>5. Person-person or faecal-oral contact</td>
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<td>6. Other risk factors (please specify)</td>
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Other important information details (e.g. treatment methods, prevention strategy):
6. Conclusions

List missing data or data that would be valuable to obtain from the authors:

Other reviewer comments:

Abbreviations:

STEC – Shiga toxin-producing Escherichia coli
HUS – Haemolytic uraemic syndrome
SD – Standard deviation
IQ – Interquartile
OR – Odds ratio
RR – Risk ratio
CI – Confidence interval
Appendix 4: Quality Assessment

NEWCASTLE-OTTAWA RISK OF BIAS ASSESSMENT SCALE FOR CASE-CONTROL STUDIES

Note: Each star represents a low risk of bias choice for each numbered item in the different categories. A study can be awarded a maximum of one star for each numbered item within each category, with the exception of comparability which can be awarded a maximum of 2 stars.

Selection

1) Definition of Controls
   a) otherwise healthy individuals *
   b) individuals with non-gastrointestinal illnesses *
   c) individuals with other gastrointestinal infection (e.g. salmonella, campylobacter)
   c) not specified

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for age (most important confounding variable) *
   b) study controls for one or more other confounding variables (e.g. geographical location or urbanisation or season) *
   b) study identifies possible confounding variables which have not been controlled for (e.g. not applied to the statistical analysis)
   c) study has not identified confounding variables

Exposure/Transmission

1) How has the study identified the exposure/transmission pathway?
   a) secure record (e.g. medical records) *
   b) structured interview/questionnaire *
   c) direct identification from outbreak data (e.g. microbiological, epidemiological)*
   d) unstructured self-report from participants
   e) no description

2) Same method of determination of exposure or transmission for cases and controls?
   a) yes *
   b) no
   c) not specified
3) Reasons given for excluding patient data?
   
a) yes (e.g. recent travel, incomplete questionnaire, inadequate diagnosis etc.) *
   
b) no

Star rating:

A maximum of 6 stars can be awarded for each study where ≥5 stars represents a study with LOW risk of bias, 3-4 stars represents a study with MODERATE risk of bias and <3 stars represents a study with HIGH risk of bias

<table>
<thead>
<tr>
<th>Risk of bias rating (HIGH, MODERATE or LOW)</th>
<th>Reviewer 1</th>
<th>Reviewer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Star rating</td>
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<tr>
<td>Risk of bias rating</td>
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</table>

Additional Comments:
NEWCASTLE - OTTAWA RISK OF BIAS ASSESSMENT SCALE FOR COHORT/OTHER STUDIES

Note: Each star represents a low risk of bias choice for each numbered item in the different categories. A study can be awarded a maximum of one star for each numbered item within each category, with the exception of comparability which can be awarded a maximum of 2 stars.

Selection

1) Selection of exposed cohort/surveillance data
   a) STEC and HUS cases from hospitals/other medical facilities *
   b) STEC and HUS cases from outbreak communities *
   c) no description/poor selection of STEC and HUS cases

Comparability

1) Comparability of cases on the basis of the design or analysis
   a) study controls for age (most important confounding variable) *
   b) study controls for one or more other confounding variables (e.g. geographical location or urbanisation or season) *
   c) study identifies possible confounding variables which have not been controlled for (e.g. not applied to the statistical analysis)
   d) study has not identified confounding variables

Exposure/Transmission

1) How has the study identified the exposure/transmission pathway?
   a) secure record (e.g. hospital records) *
   b) structured interview/questionnaire *
   c) direct identification from outbreak data (e.g. microbiological, epidemiological) *
   d) unstructured self-report from participants
   e) no description

2) Has the study considered ALL potential exposures/transmission pathways?
   a) yes *
   b) no
   c) not stated/unclear

3) Reasons given for excluding patient data?
a) yes * (e.g. recent travel, incomplete questionnaire, inadequate diagnosis etc.)

b) no

**Star rating:**

A maximum of 7 stars can be awarded for each study where >=5 stars represents a study with LOW risk of bias, 3-4 stars represents a study with MODERATE risk of bias and <3 stars represents a study with HIGH risk of bias.

<table>
<thead>
<tr>
<th>Risk of bias rating (HIGH, MODERATE or LOW)</th>
<th>Reviewer 1</th>
<th>Reviewer 2</th>
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</thead>
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<tr>
<td>Star rating</td>
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<tr>
<td>Quality rating</td>
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</table>

Additional Comments:
Appendix 5: PRISMA 2009 Flow Diagram

Records identified through database searching (n = )

Additional records identified through other sources (n = )

Records after duplicates removed (n = )

Records screened (n = )

Records excluded (n = )

Full-text articles assessed for eligibility (n = )

Full-text articles excluded, with reasons (n = )

Studies included in qualitative synthesis (n = )

Studies included in quantitative synthesis (meta-analysis) (n = )