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<th>A scoping review on the prevalence of Shiga-toxigenic Escherichia coli in wild animal species</th>
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<tr>
<td><strong>Authors(s)</strong></td>
<td>Espinosa, Laura, Gray, Aimée, Duffy, Geraldine, Fanning, Séamus, McMahon, Barry J.</td>
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A scoping review on the prevalence of Shiga-toxigenic \textit{Escherichia coli} in wild animal species

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ABSTRACT

Zoonotic pathogens constitute the major source (60.3%) of emerging infectious diseases. Previous studies have investigated the prevalence of Shiga-toxigenic Escherichia coli (STEC) among wild animal species, but comprehensive data is needed to assess the role that these animals have in the transmission of STEC infections to the human population via faecal contamination of the environment, agri-food or water chain. Due to the nature of these microorganisms in which this human-animal-environment interface plays a relevant role on the disease’s dynamics, a ‘One Health’ approach is needed to prevent and control the worldwide spread. The aim of this paper is to review the published research on the prevalence of STEC in wildlife. The search was performed using several online databases consisting of three blocks of specific search terms covering pathogen, type of study and population. Two reviewers applied the inclusion and exclusion criteria to screening and eligibility phases. Two-hundred twenty-five abstracts were screened for relevance and 72 were included for data analysis. Most studies (77.8%) investigated the prevalence of STEC in ruminants and urban birds. Their role in transmitting the pathogen to humans, other animals and the agri-food chain is potentiated by the peculiar biological characteristics in ruminants and improved adaptation of urban birds to urban environments. The popularity of convenience and voluntary response sampling may be due to the lack of human-made boundaries on the wild animal species’ habitat and having some samples from hunted-harvested animals. To our knowledge, this is the first comprehensive review on STEC prevalence in wild animal species from studies conducted across the globe. We recommend that future research includes and compares samples from varying origins (i.e. human, animal, environment, and food) and applies a ‘One Health’ approach to the emerging challenges that STEC poses to public health.

KEY WORDS

Birds, Deer, One Health, Shiga-toxigenic Escherichia coli, Zoonoses.

IMPACTS

• This scoping review provides a worldwide mapping of the published research on prevalence of STEC in wild animal species.
• Ruminants and urban birds are the predominant wild animal species sampled in the studies with a median prevalence of STEC of 4.7% and 1.2%, respectively, among the total number of samples analysed
• Only 10% of the 79 studies included for data extraction investigated the prevalence of STEC in wild animal species in conjunction with livestock and humans; which does not reflect the suggested One Health approach to tackle infectious diseases such as STEC
1. INTRODUCTION

Zoonotic pathogens constitute the major source (60.3%) of emerging infectious diseases (Jones et al., 2008). In the last decade, 72% of zoonotic emerging infectious diseases were caused by pathogens with a wildlife origin, with an increasing trend since late 20th century (Jones et al., 2008). In addition, urbanisation and adaptation of urban exploiter species such as rats (*Rattus* spp.) or pigeons (*Columba* spp.) in urban areas have increased contact between certain wild animal species and people, potentiating the transmission of zoonotic pathogens (Rothenburger, Himsworth, Nemeth, Pearl, & Jardine, 2017) via faecal contamination of the environment, agri-food or water chain.

*Escherichia coli* is part of the normal gut flora of humans and animals and can be transmitted among different species. Although most *E. coli* are commensal organisms, there are a number of pathotypes which can cause a variety of illnesses in humans. The most noteworthy are enterohemorrhagic *E. coli* (EHEC) which can cause severe disease in humans and are considered the most frequent cause of haemolytic uremic syndrome. These EHEC pathotypes are a subset of Shiga toxigenic *E. coli* (STEC), also known as verocytotoxigenic *E. coli* (VTEC), which present Shiga toxin 1 or 2 gene (*stx*1 or *stx*2) (Croxen et al., 2013). Despite STEC O157 with serotypes H7 or H are the most common serogroups of EHEC, other serogroups have been associated to HUS outbreaks including, but not only, O26, O45, O103, O111, O121 and O145 (Kuehne et al., 2016).

While ruminants are recognized as a principal reservoir for STEC, preweaning calves rarely carry STEC possibly as a consequence of their diet, their non-ruminant gastrointestinal tract and the management system (Gyles, 2007). On the contrary, ruminating postweaning calves and heifers present much higher prevalence than older cattle (Ferens & Hovde, 2011).

STEC infection in ruminants varies according to age of the animal and status of the gastrointestinal tract (i.e. ruminating or non-ruminating), as previously described, and typically has intense periods of shedding interspersed with periods of non-shedding. Some animals are known to be “super-shedders” (shedding >10^4 CFU/g feces over long periods) and such animals are thought to be a significant contributor in the dissemination of STEC O157 into the environment and its transmissibility (Ferens & Hovde, 2011).

As part of the International Health Regulations (IHR), countries must report to WHO any disease event that may constitute a public health emergency of international concern, and STEC outbreaks are reported following this international legal instrument (World Health Organization, 2016a). Moreover, notifying data on cases of STEC infections in humans and STEC detected in food and food-production animals is mandatory in some regions of the world such as the European Union and European Economic Area (European Centre for Disease Prevention and Control and European
Food Safety Authority, 2011) and the United States of America (Centers for Disease Control and Prevention, 2017; United States Department of Agriculture, 2017).

An increasing number of STEC outbreaks, mainly but not only due to the serogroup O157, are associated with the consumption of fruits and vegetables that may result from contamination with domestic or wild animal faeces (World Health Organization, 2016b). For example, a large outbreak of Escherichia coli O157:H7 was reported in the US in 2011 and the investigations identified fresh strawberries as the vehicle and deer faeces as the source of contamination (Laidler et al., 2013).

Previous studies investigated the prevalence of STEC among urban exploiter species such as rats (Himsworth et al., 2015) or pigeons (Gargiulo A. et al., 2014; Kobayashi, Pohjanvirta, & Pelkonen, 2002; Murakami et al., 2014; Silva, Nicoli, Nascimento, & Diniz, 2009). Likewise, other wild animal species have been investigated such as deer (Asakura et al., 2017; Carrillo-Del Valle et al., 2016; Dunn, Keen, Moreland, & Alex, 2004) and gulls (Makino et al., 2000). Nevertheless, more comprehensive data on the prevalence of STEC in wild animal species is needed to better assess the role that these animals have in the transmission of STEC infections in the human population and food chain. This knowledge would support decision-makers in the prioritisation of public health interventions to prevent and control STEC infections in humans and to manage exposure to this pathogen from the agri-food chain (Allin, Mossialos, McKee, & Holland, 2004).

The aim of this study is to review the published research on the prevalence of STEC in wildlife, with a particular focus on deer and gulls, which can be used to identify specific topics for targeted systematic reviews or support public health interventions to prevent/control the transmission of this pathogen to humans and the food chain.

2. METHODS

This scoping review was based on the framework outlined by Arksey and O’Malley (Arksey & O’Malley, 2005). The following steps were followed: definition of the research question; identification of the relevant studies; study selection; charting the data; collation, summary and reporting of the results. The additional optional step of expert consultation (Levac, Colquhoun, & O’Brien, 2010) was not performed in this review.

2.1. Defining the research question

The following research question was investigated: ‘What is the prevalence of Shiga-toxigenic Escherichia coli (STEC) in wild animal species?’.
2.2. Study identification

The search was performed during June 2017 in several online databases: Medline/Pubmed, Scopus, Cochrane, Lilacs, ScieLo and Google Scholar. In addition, references from the included studies and previous reviews were screened for additional studies.

The search strategy consisted of three blocks of specific search terms covering pathogen (e.g., ‘Shiga-toxigenic *Escherichia coli*’ or ‘*Escherichia coli O157*’), type of study (e.g. ‘prevalence study’ or ‘case study’) and population (e.g. ‘wild animal’ or ‘gull’), joined by the Boolean operator ‘AND’. The search strategy was tailored to the specific requirements of each database (available in the supporting information).

All citations were imported into open-source bibliographic manager Zotero version 5.0.27 (Center for History and New Media, 2017) and imported into Covidence Systematic Review software trial version (Veritas Health Innovation, 2017). This software eliminated duplicated studies automatically for the screening and allowed an interactive and parallel screening and eligibility assessment from all reviewers.

2.3. Study selection

Inclusion and exclusion criteria were preliminary established based on the research question, were revised *post hoc* based on increasing familiarity with the literature and then applied to all citations. The inclusion criteria used in this scoping review were related to population (wilde animals or non-captive wild animals), pathogen (*Shiga-toxigenic Escherichia coli*, *verotoxigenic E. coli*, and known serogroups of Shiga-toxigenic/verotoxigenic *E. coli* such as O157), type of study (survey, cross-sectional study, cohort study, case-control study, outbreak investigation and review study) and language (English, Spanish, French, Italian and German). The exclusion criteria were related to population or sources (captive animal, livestock, pets, zoo animals, food products or experimental animals), pathogen (non-*Shiga*-toxigenic/non-*verotoxigenic E. coli*), type of study (experimental studies or microbiological studies evaluating detection methods) and language (other languages not mentioned in the inclusion criteria). Studies were also excluded if there was no free accessible full-text in open-source journals, online repositories or library catalogue from University College Dublin. There was no limitation on location nor year of publication.

Two reviewers applied the inclusion and exclusion criteria (LE and AG) to screen titles and abstracts of the identified records. Full-texts were retrieved from the selected records to apply the same inclusion and exclusion criteria for the eligibility assessment. Disagreements between the reviewers were discussed until an agreement was reached.
2.4. Data extraction

Data was extracted and charted into Microsoft Excel including information on author, year of publication, country, type of study, wild animal species, type of sampling, starting year of sampling, number of samples, test method to detect STEC, and number of STEC positive samples.

Completeness on the data concerning methods, number of samples and proportion of STEC positive samples was evaluated. Studies with missing data or referring to other included studies were eliminated for the data analysis.

2.5. Data analysis

Descriptive analysis of the data was performed with R version 3.4.2 (R Core Team, 2017); including frequency and proportion of different characteristics of the studies, number of samples analysed, and proportion of STEC by animal category, study location, sampling type and starting year of sampling. For convenience reasons, wild animal species were grouped into seven categories (hereafter referred as animal categories) according to their ecology and number of studies that included these species (described below).

3. RESULTS

3.1. Study search and selection

The flow diagram of citations is shown in Figure 1. Two-hundred twenty-five abstracts were screened for relevance. Full-text was obtained from 96 articles for eligibility assessment and 79 studies were included for data extraction (available in the supporting information). Seven of the studies were excluded from the data analysis due to lack of specific data (n = 4), incomplete data on number of samples (n = 2) or lack of data on proportion of STEC (n = 1). Twenty studies evaluated the prevalence of STEC in different wild animal species.

3.2. General characteristics of included studies

The general characteristics extracted from the 72 studies are summarised in Table 1. More than 70% of the studies were published since 2000 and one quarter of those (13 studies) were published in the last three years. Countries were grouped in regions following the United Nations geo-scheme (United Nations, 1999), and the most common study location was North America (37.5% of the 72 studies) followed by Europe (36.1%), Asia (19.4%) and Central and South America (7.0%). No study from Africa or Oceania was found. Surveys were the predominant type of study (96.2% of the studies analysed).
According to ecology and frequency, wild animal species were grouped into seven categories namely ruminants, rodents, other mammals, urban birds, non-urban birds, other wild animal species or vectors, and unspecified or unknown species. Table 2 shows wild animal species that were investigated in the 72 studies grouped by animal category including those in which STEC was not found. Some studies investigated more than one wild animal species and/or animal category, so the total sum of proportion is more than 100%. Most studies (77.8%) investigated the prevalence of STEC in ruminants and urban birds, with a specific predominance of deer (41.7%) and pigeons (23.6%).
Table 1. General characteristics of 72 studies included in the data analysis, showing the quantity of studies (No.) and the proportion of the total 72 studies (%) with each specific characteristic.

<table>
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<tr>
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<th>%</th>
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<td><strong>Total</strong></td>
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<td>72</td>
<td>100</td>
<td>Total</td>
<td>72</td>
<td>100</td>
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</table>

† China, India, Iran and Japan.
‡ Mexico, and Trinidad and Tobago.
§ Austria, Belgium, Denmark, England, Finland, Germany, Italy, Poland, Spain, Sweden and Switzerland.
¶ Canada and United States of America (USA).
†† Brazil and Peru.

3.3. Sampling and laboratory protocols

Convenience was the most frequent type of sampling, performed in half of the studies; and the starting year of sampling was between 1995 and 2004, both inclusive, in 56% of the studies. PCR was the most common technique (62.5%) to detect STEC, followed by agglutination test (18.0%) and agglutination combined with other tests (9.7%) (Table 1). The vast majority of the studies (89.0% of the 72 studies) tested for STEC in isolates retrieved from the wild animal samples and the remaining 11.0% performed the testing from enrichment broth of the wild animal samples. There was no statistically significant difference in the proportion of STEC positive samples according to the laboratory test used for detecting STEC. However, a noticeable variation of proportion of STEC positive samples within each technique was observed with a IQR of 40.0%, 8.9%, 8.0%, 2.8% and 2.3% in studies using ELISA, PCR, combined techniques, agglutination and Vero cell cytotoxicity assays, respectively, to analyse the proportion of STEC positive samples.
Table 2. List of wild animal species investigated in the 72 studies, grouped by category of wild animal species; showing the quantity of studies (No.) and the proportion of the total 72 studies (%) in which each wild animal species was studied. Some of these studies investigated more than one wild animal species and/or more than one animal category, so the total doesn’t sum up 100%.

<table>
<thead>
<tr>
<th>Wild animal species</th>
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<td>4.2</td>
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<td>2.8</td>
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</tr>
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<td>Fox</td>
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<tr>
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<tr>
<td>Agouti</td>
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<tr>
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<tr>
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<td>Raccoon</td>
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</tr>
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<tr>
<td>Unknown</td>
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<td>2.8</td>
<td>Flies</td>
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<tr>
<td></td>
<td></td>
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<td></td>
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<td>Fish</td>
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The number of samples varied from one study to another, ranging from 1 to 2,084 samples in total without stratifying by animal category. It also differed according to wild animal species. Figure 2 shows a boxplot of the number of samples analysed by animal category. Rodents showed the highest variation, ranging from 7 to 1,116 samples.
### 3.4. Prevalence of STEC in wild animal species

The prevalence of STEC in wild animal species was investigated in conjunction with a livestock sharing environment (38% of the 79 studies), as part of an outbreak investigation (2.5%) or solely in wild animal species (60%).

A stratified analysis by animal category on the proportion of STEC positive samples reported in the 72 studies was performed. Figure 3 shows the proportion of STEC positive samples by animal category, study location, sampling type and starting year of sampling. The prevalence of STEC varied from one animal category to another, with an overall median of 1.7% and an IQR of 0-8.8%. Ruminants had the biggest variation on proportion of STEC positive samples per study with a median of 4.4% and interquartile range of 0.3-20.5%. Across regions of the world, Europe had the highest median of STEC prevalence (6.2%) and highest variation with an IQR of 1-12.5%.

Likewise, a stratified analysis on the proportion of STEC positive samples by sampling type and number of samples analysed was performed. There was no noticeable difference on the STEC prevalence among different sampling types.
When comparing the proportion of STEC positive samples with number of samples analysed (Figure 4a), a negative trend could be observed with higher prevalence when a smaller number of samples was analysed. Similarly, considering that in general at least 30 samples are required for further statistical analysis, a comparison was done among the proportion of STEC positive samples with number of samples analysed of those studies that at least analysed 30 samples (Figure 4b), and the trend was slightly less noticeable. Furthermore, if a stratification by animal category was done in this last analysis, only ruminants (Figure 4c) and urban birds (Figure 4d) showed a negative and positive trend, respectively, and the rest of species appeared to have no relation among those two variables.

4. DISCUSSION

In this scoping review, we identified the published worldwide research on the prevalence of STEC in wild animal species and synthesised the main findings in a transparent and systematic manner. To our knowledge, this is the first comprehensive review on prevalence of STEC in different wild animal species and world regions.

Zoonotic pathogens are the major cause of infectious diseases in humans (Jones et al., 2013; Jones et al., 2008; Woolhouse & Gowtage-Sequeria, 2005). Due to the nature of these microorganisms in which the human-animal-environment interface plays a relevant role on the disease’s dynamics, a ‘One Health’ approach is needed to prevent and control the worldwide spread (Rüegg et al., 2017).
STEC and other *E. coli* are an example of zoonotic pathogens with an increasing concern due to emerging challenges to public health as a consequence of changes in the pathogen, such as strains expressing higher levels of certain virulence factors; population and environment (Karmali, 2017). Existing studies have investigated the role and risk factors of livestock and other domestic animals in the dynamics of STEC infection in humans. Nevertheless, there is limited published research on the role of wild animals in the transmission of STEC to humans, domestic animals and within the food chain. We found that the number of publications has been increasing since the mid-2000s; yet, comprehensive reviews presenting the status of the prevalence of STEC in specific wild animal species and locations are lacking.

Similarly, the proportion of STEC in wild animal species also increased since 2000s according to the starting year of sampling. It can be explained by changes of anthropogenic nature such as human population growth which approximates drivers of disease emergence including increased interspecies contacts between humans, domestic animals and wild animal species (Engering, Hogerwerf, & Slingenbergh, 2013). This emergence of STEC in wild animal species could potentially have an opposite spill-over or ‘spill-back’ in domestic animals (Daszak, Cunningham, & Hyatt, 2000), creating a circle of transmission that increases the prevalence of this pathogen not only in wild animal species but also in human and other animal populations. A recent systematic review on the global incidence of human STEC infections (Majowicz et al., 2014) shows that Eastern Mediterranean countries had the highest estimated incidence in 2012 and African countries had the lowest estimated
incidence. Contrasting, our review, found that North America was the region with the highest prevalence of STEC in wild animal species and we did not uncover any studies carried out in African countries. Moreover, most of the studies were performed in North America (37.5%) or Europe (36.1%) showing the commitment of these regions to the monitoring of diseases in wild animal species.

The popularity of convenience and voluntary response sampling may be due to the lack of human-made boundaries on the wild animal species’ habitat and having some samples from hunted-harvested animals (Mörner, Obendorf, Artois, & Woodford, 2002). Despite the recommendation to combine these sampling methods with more systematic sampling methods (Nusser, Clark, Otis, & Huang, 2008), this review found that the median prevalence stratified by sampling type were similar, concluding that a superior sampling method is not evident.

When performing convenience and voluntary response samplings, sampling size cannot be determined in advance with systematic methods. There was wide variation on the number of samples analysed in the 72 studies, ranging from 1 to 2,084 samples. When plotting the number of samples analysed and the proportion of STEC positive samples by animal category and discarding those studies with less than 30 samples analysed, a noticeable relationship was visible in ruminants and urban birds. Due to the heterogeneity of data, this tendency is not statistically significant. However, it is relevant to mention that these animal categories had the highest proportion of convenient sampling (45.5% and 47.8% of the total number of studies including ruminants and urban birds, respectively) which could explain this tendency that should not be present if there was no bias in the sampling strategy.

There was no statistically significant difference among laboratory techniques used in each study and prevalence of STEC. However, the higher variation in the results from studies using ELISA and lower variation from those using PCR is a consequence of the higher accuracy of PCR as STEC diagnostic test in comparison to commercially available immunoassays (Pulz et al., 2003).

Overall, the median of STEC prevalence by animal category was similar whereas there was a noticeable difference in the variability of STEC prevalence among animal categories. This variation was significantly higher in ruminants in comparison with the other animal categories.

Ruminants were the most common wild animal species investigated in the analysed studies, with deer dominating the literature (41.7% of the 72 studies). Likewise, ruminants had the highest median of STEC prevalence (4.7% of the analysed samples were STEC positive) among all animal categories. This corresponds with their peculiar biological characteristics (i.e. lacking the vascular receptors to the stx toxins) that make them less susceptible to the pathogen and increased potential of excretion in the environment. There was a noticeable variation in the prevalence of STEC in ruminants among studies with only four studies showing prevalence higher than 50% and, in contrast,
with five studies showing absence of STEC. This wide range of STEC proportion can be explain, for example, with different sampling sizes (e.g. the study that showed 100% prevalence of STEC investigated only five deer samples) or year of sampling (e.g. studies showing absence of STEC were performed in the late 90s) since STEC prevalence in wild animal species is increasing since the 2000s.

Urban birds were the second animal category most commonly investigated (31.9% of the 72 studies), despite having the fourth higher median of STEC prevalence among all animal categories. These wild animal species, as well as other urban exploiter species, are well adapted to urbanised ecosystems where inter-relation with humans is potentially increasing the transmission of infectious diseases, including STEC, which can explain being the second animal category most commonly investigated.

Urban birds and rodents were among the animal categories with less variation in the STEC prevalence within each category. It can be explained by the fact that these animal categories are easier to be sampled possibly due to being close to anthropogenic environments (e.g. cities or farms) which allows to have higher sampling sizes and more accurate results.

Nevertheless, transmission of infectious diseases from wild animal species to humans is not always due to direct contact with the infected animal. A recent study of an STEC outbreak in eastern England attributed causation to wild rabbit faeces from a wildlife park as the route of infection to humans and found that these rabbits were in contact with STEC-positive cattle in an adjacent field (Crook, Senior, & Senior, 2017). Moreover, flies are recognised as mechanical vectors of STEC (Puri-Giri, Ghosh, Thomson, & Zurek, 2017) and our review showed that flies had the second highest median prevalence of STEC among the 72 studies included for data analysis.

In line with the aim of this review, most of the studies (65% of the 72 articles included for data analysis) investigated the prevalence of STEC in deer and gulls, independently of any other species or sources, and almost all studies (95.8%) were surveys. For this reason, a more systematic and comprehensive methodology, such a systematic review, on the published research about prevalence of STEC in deer and/or gulls could produce strong baseline knowledge to support decision-makers in prioritising public health interventions to prevent and control STEC infections in animals, humans and food chain.

The ‘One Health’ initiative recommends multi-discipline collaboration for an increase in sustainable and cost-effective approaches in preventing infectious diseases as well as other public health interventions (Queenan et al., 2017). Likewise, the new Sustainable Development Goals published by WHO in 2015 (World Health Organization, 2015), stresses the necessity of an integrated approach across environmental, economic and social pillars to address all infectious diseases.

In the 79 studies included in this review for data extraction, less than half (40.4%) investigated the prevalence of STEC in wild animal species in relation to livestock, environment or humans; and
only 25% of those (eight studies) investigated the prevalence of STEC in wild animal species in conjunction with livestock and humans. These results do not reflect the suggested multidisciplinary and integrated approach to tackle infectious diseases such as STEC. We strongly recommend that future research addresses this gap by including and comparing samples from varying origins (i.e. human, animal, environment, and food) and to have a more comprehensive approach to the emerging challenges that STEC poses to public health.

4.1. Strengths and limitations of this scoping review

There is a large number of recently published research on different aspects of STEC in livestock including studies on risk factors (Lee, Kusumoto, Iwata, Iyoda, & Akiba, 2017; Suardana, Widiasih, Nugroho, Wibowo, & Suyasa, 2017), nationwide survey on STEC prevalence (Henry et al., 2017), factors that increases faecal shedding in ruminants (Stenkamp-Strahm et al., 2017) and strategies for reducing the risk in animals (Saeddi et al., 2017). However, to our knowledge, this is the first comprehensive review on STEC prevalence in wild animal species from a global perspective.

The optional step of expert consultation was not performed because of lack of adequate resources. Nevertheless, all references from included studies were screened for additional records (n = 34) and 25 of those were included in the data extraction. Likewise, Google Scholar was included in the search strategy trying to retrieve grey literature.

5. CONCLUSIONS

The scoping review methodology (Arksey & O’Malley, 2005) created a useful overview of the available research on a specific topic, in this case, STEC prevalence on wild animal species. The results from this review have allowed to pinpoint specific areas for a future systematic review (i.e. surveys on STEC prevalence in deer and/or gulls) as well as provided a solid knowledge that can be used to prioritise public health intervention towards reducing this infection in humans, animals and the food chain. Despite having strong evidence on the role of wild animal species in the transmission of STEC infections to human, other animals and within the food chain (Crook et al., 2017), there is a gap on more comprehensive research on the ‘One Health’ perspective of this public health challenge, e.g. investigating STEC in different sources and comparing results in order to find routes of infection and targets for control/prevention interventions. Integrating the human-animal-environment interface in STEC studies will allow more comprehensive and cost-effective strategies to prevent and control these challenging infections.
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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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Espinosa et al. Prevalence of STEC in wildlife: scoping review


### SUPPORTING INFORMATION 1. DETAILS OF SEARCH STRATEGY.

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SUPPORTING INFORMATION 2. LIST OF THE 79 STUDIES INCLUDED FOR DATA EXTRACTION.


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https://doi.org/10.1292/jvms.64.1071


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