CREW CENTRE OF EXPERTISE FOR WATERS

The epidemiology and disease burden potential relating to private water supplies in Scotland

Appendices

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CREW CENTRE OF EXPERTISE FOR WATERS

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Appendix I. *Salmonella*

1.0 Hazard identification

1.1 The organism

Salmonella spp. are rod-shaped bacteria (size approximately 0.7µm by 2 - 5µm), members of the family Enterobacteriaceae, which cause typhoid fever, paratyphoid fever and gastroenteritis. The genus comprises two species, *Salmonella bongori* (very rare in the UK) and *Salmonella enterica.* The later contains more than 2500 serovars with *Enteritidis* and *Typhimurium* being the two main serotypes found in the UK (Lane et al. 2014; Mindlin et al. 2013).

1.2 Growth and survival

Salmonella grow under the following conditions outside living hosts. They can grow in the presence of 0.4 to 4% sodium chloride. Most *Salmonella* serotypes grow at temperature range of 5 to 47°C. The optimum growth occurs at 35 to 37°C and some strains can grow at temperatures as low as 2 to 4°C or as high as 54°C (Pui, Wong et al. 2011). *Salmonella* grow in a pH range of 4 to 9 with the optimum between 6.5 and 7.5. They need high water activity (a_w) between 0.99 and 0.94 to grow but can survive at a_w <0.2.

1.3 Inactivation (Critical Control Points and Hurdles)

Salmonella are killed at temperatures of 70°C or above (Bhunia 2008; Hanes et al. 2003). Complete inhibition of growth occurs at temperatures <2°C, pH <3.8 or water activity <0.94 (Bhunia 2008; Hanes et al. 2003; Pui et al. 2011). A four log reduction is achieved when applying 13 -18 Jml^{-1} UV-C light (Gayan et al. 2012) and additional data are provided for *Salmonella* in Table 8 in the main report.

1.4 Sources

Salmonella spp are found in both cold- and warm-blooded animals and are widely distributed in the environment. Serovars including *S. Typhimurium* and *S. Enteritidis* are prevalent in animals including poultry, pigs, cattle, sheep and wild birds (Barrow & Methner 2013). In the UK vaccination against *S. Enteritidis* in breeder chicken flocks was introduced in 1993 (Lane et al. 2014). Bayesian analysis on surveillance data of *Salmonella* occurrence in flocks of laying hens in the UK indicated a flock prevalence of 14% for *S. Enteriditis* and S. *Typhimurium*, and 18% for all serovars (Arnold et al. 2010). An abattoir study in 2003 showed a 23.4% (95%CI, 19.9-27.3) prevalence of *Salmonella* in pigs from UK (70% of positive being *S. Typhimurium*) (Berriman et al. 2013). During a 24 weeks longitudinal study on six dairy cattle farms in UK, following prior identification of S. *Typhimurium*, between 6.8% to 75% of the faecal samples were found to be positive for S. *Typhimurium* and concentrations of up to 10^7 cfu/g of faeces were detected (Kirchner et al. 2012). Another study at 93 abattoirs in the UK found a 1.7% *Salmonella* prevalence in cattle faeces and 1.1% in sheep faeces, respectively (Milnes et al. 2008). Data on prevalence of *Salmonella* in wild birds is limited. However, between 2.7 to 8.7% of black-headed gulls were found positive for *Salmonella spp* in Sweden (Palmgren et al. 2006). The intestinal tract of vertebrates is presumed to be the native habitat of *Salmonellae* (Woodward et al. 1997). Drinking waters are infected via sewage discharge and runoff from livestock faeces and from faeces of wild animals (Levantesi et al. 2012).

2.0 Hazard characterisation: adverse health effects

2.1 Disease symptoms

Salmonella cause self-limiting gastroenteritis with symptoms that include diarrhoea, nausea, vomiting, fever and abdominal cramps. Most severe sequelae include bacteraemia or septicaemia and typhoid/enteric fever. For non-typhoid gastroenteritis the symptoms typically can last between 6 to 72 hours and diarrhoea can last up to 5 days. In developed countries, where the sanitary systems are good such as in the UK, typhoid fever is uncommon.

2.2 Dose response

In this subsection two mathematical dose-response models (exponential and Beta-Poisson) used in quantitative risk assessment for pathogenic micro-organisms are presented (Teunis et al. 1999a). In summary these two models predict the probability of infection following ingestion of one or more pathogenic micro-organisms. Both models assume that the chance of infection increases with the number of organisms ingested. The selection of which model to use is dependent upon the best fit of the models to data. Once the best fit model has been found it is relatively easy to implement into a risk assessment model. For *Salmonella* it was found the dose at which there is a 50% chance of infection is approximately 7 organisms.

The details of the models are provided below.

The exponential model (EM)

The exponential dose response model is a single hit model where only one organism is required to cause infection and all organisms act independently. Given a dose of D organisms that are Poisson distributed and given that each organism has a probability r of surviving to cause infection then the probability (P_{Inf}) of the host becoming infected can be calculated from:

$$P_{Inf} = 1 - e^{-rD} \quad (1)$$

The maximum likelihood method can be used to fit this model to dose response data and from this the best fitting value for r can be obtained and used to calculate the dose necessary for 50% of the population to become infected:

$$ID_{50} = \frac{\ln(0.5)}{-r} \quad (2)$$

The approximate Beta-Poisson model (B-P)

Both host and pathogen heterogeneity can be incorporated using a beta distribution B (α, β) to describe the probability r of a pathogen surviving the barriers within a host to cause infection. The probability of the host becoming infected becomes:

$$P_{Inf} = 1 - \int_0^1 e^{-rD} B(\alpha, \beta) dr \quad (3)$$

Integrating over r when β>>1 and α<<β results in the approximate Beta-Poisson model:

$$P_{Inf} = 1 - \left[1 + \frac{D}{\beta}\right]^{-\alpha} \quad (4)$$

Again, the maximum likelihood method can be used to fit this model to dose response data and from this the parameters α and β can be determined. The corresponding ID_{50} can be expressed as:

$$ID_{50} = \beta[2^{1/\alpha} - 1] \quad (5)$$

Dose response data for *Salmonella* from outbreaks in humans is available from the literature (Kothary & Babu 2001; McCullogh and Eisele 1951a; McCullogh & Eisele 1951b). There have been several attempts to fit these data using the exponential model, the Beta-Poisson or other models (Haas 1983; Teunis et al. 1999a; Latimer et al. 2001; Holcomb et al. 1999).

The Beta-Poisson model developed by Teunis and colleagues (Teunis et al. 2010) is arguably the most complete, as it used data from outbreaks of salmonellosis (*S. Enteritidis* or *S. Typhimurium*) and not from feeding studies, which can be biased by the types of strains used for challenging the human subjects or by the fact that only healthy adult volunteers are being challenged. The following fit parameters (a and b) and corresponding ID_{50}s were obtained: $\alpha = 8.53 \times 10^{-3}$, $\beta = 3.14$ (ID50=6.65 (95% CI – 0.69 – 5.89 x 10^4)).

2.3 Susceptible population

There are a number of predisposing factors (conditions or comorbidities) which can increase the risk of contracting *Salmonella*. These include frequent use of antibiotics, chronic steroid administration, HIV infection, impaired immune system, cancer, sickle cell disease, ulcerative colitis, malnutrition, inflammatory bowel syndrome (Magni 2010). The elderly population (>65 years old) and senior adults (36-65 years old) were at higher risk in Spain (>84% of cases recorded in a hospital between 1991 to 2001) (Rodriguez et al. 2006). In Scotland the incidence of salmonellosis is highest in young children (<5 years) and the elderly (>65 years) https://tinyurl.com/t6xp6tn . In the USA women were at higher risk for *Salmonella* bacteriuria (70% of *Salmonella* urinary isolates reported between 1980 to 1999), particularly in <1 year old and >70 years old (Sivapalasingam et al. 2004).

2.4 Particular subtypes found in both humans and PWS

At the time when this review was written there were only three references in the scientific literature (web of knowledge) related to "*Salmonella* and outbreaks and water" in the developed world. First an outbreak study in the USA (Anonymous 1971) identified *Salmonella typhimurium* (phage type 2) as the causative agent of disease (i.e. it was found both in the municipal drinking water during the outbreak and faecal samples from patients). Second *Salmonella typhimurium* was involved in another USA outbreak, which was related to municipal water consumption (Ailes et al. 2013). Third in Spain, *Salmonella Kottbus* caused an outbreak in infants due to consumption of bottled water (Palmera-Suarez et al. 2007). Also, there was one epidemiological study from Canada on sporadic cases of *Salmonella*, which found no association between occurrence of the disease and drinking from PWS (Levallois et al. 2014). In Scotland there has been 41 *Salmonella* outbreaks in the ten- year period (2005-2014) where none were associated with drinking water (Anonymous 2016).

3.0 Exposure assessment

3.1 Contamination prevalence/ frequency, concentration, survival/ growth in water

There are no data on the prevalence of *Salmonella* in PWS in Scotland and in the rest of the UK. There are also no data on survival in drinking water in the UK. However a study of *Salmonella* survival in bottled mineral water indicates that the pathogen did not grow and a reduction was observed (Ramalho et al. 2001). Survival in fresh lake water indicates that the die-off (decay) coefficient increases with the increase in temperature, varying from approx. 0.2 $\log_{10}$ /day at 4°C to approx. 1.0 $\log_{10}$ /day at 20°C (Pachepsky, Blaustein et al. 2014). This improved survival at lower temperatures has also been observed for *E. coli* O157 and *Campylobacter* (see subsections 5.4.2.2 & 5.4.2.3 in the main report). There appear to be no studies of temperature in PWS. However, in the river Spey the temperature varies between 5 and 16°C during the year (Pohlel et al. 2019).

3.2 Dose ingested

There is no information on the dose of *Salmonella* ingested from drinking water from PWS in Scotland or the UK.

4.0 Risk characterisation

4.1 Incidence

The incidence of *Salmonella* between 2001-14 for the Scottish population is given in Figure A.1 (Browning et al. 2015). It has declined from 31 cases/100,000 in 2001 to 13.5 cases/100,000 in 2014.

4.2 Clinical consequences of infection

There is a lack of specific data in the literature on the clinical consequence of salmonellosis infection in Scotland. However, based on an English survey (Adak et al. 2002) approximately 3.6% of reported cases are hospitalised and 0.29% end in death. These figures are lower than the most recent findings from a Spanish study (Gil Prieto et al. 2009) which reported a mortality rate of 1.4%. This study also reported that mortality increases with age (e.g. 7.5 % for people >85 years old).

4.3 Outbreaks

In the 10 years from 2005 to 2014 there were 41 *Salmonella* outbreaks in Scotland which were reported to Health Protection Scotland (Anonymous 2016). These outbreaks accounted for approximately 562 cases (~13.7 cases/outbreak) and none were waterborne related.

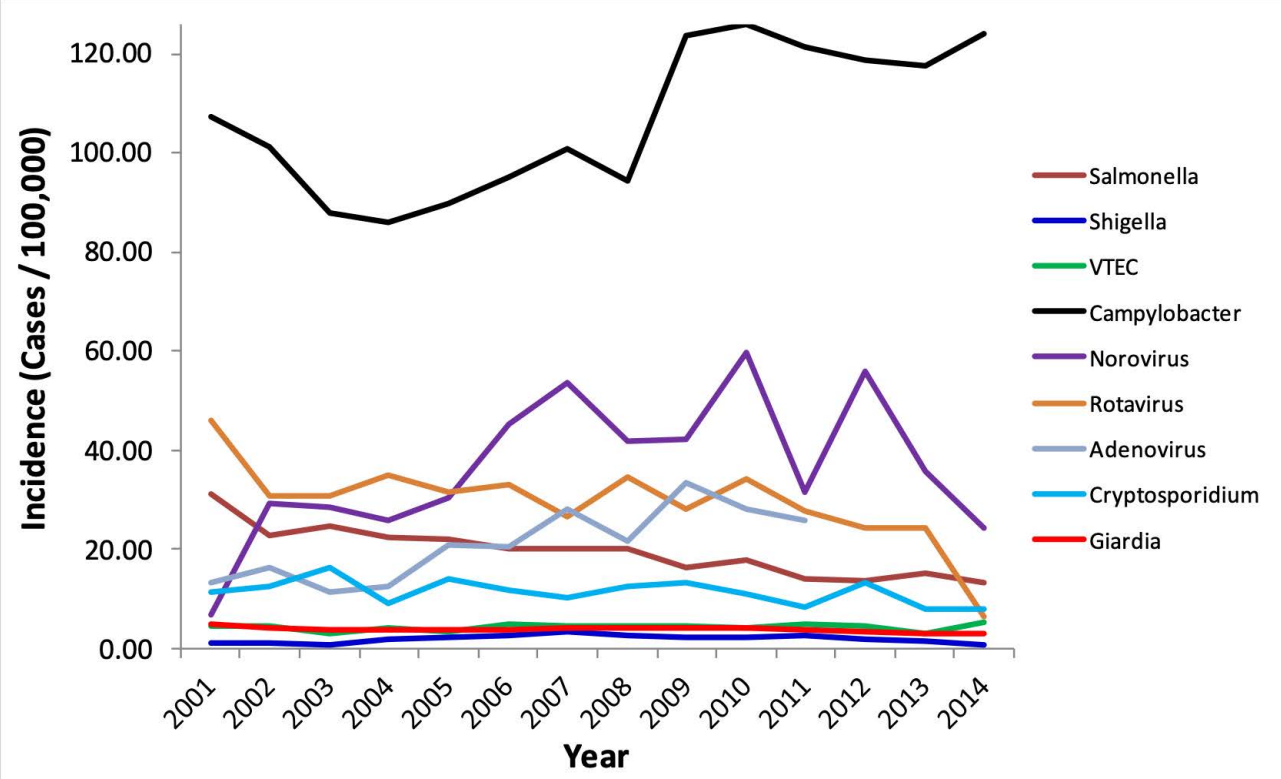

Figure A.1 The disease incidence of human cases for the pathogens considered in this study (Salmonella, Shigella, VTEC, Campylobacter, Norovirus, Rotavirus, Adenovirus, Cryptosporidium and Giardia).

4.4 Sporadic cases

There has been no study (e.g. case-control) which has investigated whether sporadic cases of salmonellosis in Scotland are associated with PWS. A case-control study from Canada on gastroenteritis of putative drinking water origin considered *Salmonella*, but found no significant difference between GI cases and controls for PWS (Levallois et al. 2014).

4.5 Risk assessments

There are multiple ways of assessing or giving an understanding of risk. These can include: comparison of disease incidence and/or disease severity between pathogens; case-control and case-case studies which identify risk factors (e.g. PWS) and determine population attributable fractions; quantitative microbial risk assessments which give the probability of risk and estimate the number of cases; attribution models using genetic/phenotypic data about pathogens from both clinical and sources of contamination which determine the proportion of disease cases attributable to a specific source.

There has been no qualitative or quantitative assessment of risk for waterborne salmonellosis either in Scotland or the rest of the UK. A risk assessment has been developed in Thailand for swimming in surface waters, which were also water sources for treatment plants supplying tap water for metropolitan communities (Banmairuroy et al. 2014). As mentioned above illness from swimming is out of scope in this report. However, the reference has been kept in as it provides an example of a QMRA for *Salmonella*.

4.6 Qualitative/Quantitative estimate of risk

There are no available risk assessments for *Salmonella* in Scotland.

4.7 Risk categorisation

The rationale for categorisation of PWS/hazard combinations is presented in Appendix VI.

Disease incidence

The burden of disease caused by drinking water from private water supplies is unknown. There are also no *Salmonella* models which determine the incidence of disease by transmission pathway, source, or any other risk factor (e.g. food, drinking water from PWS, etc.). Therefore, the overall incidence, from all sources, for each pathogen is used for risk categorisation (see Table A1 in Appendix VI). *Salmonella* together with *Rotavirus* and *Adenovirus* were categorised as medium risks (rank 3) in terms of incidence.

Disease severity

The severity of disease from waterborne infections is also unknown. This is also required for risk categorisation but has not been done here. However, if it is assumed that the spectrum of disease obtained from all cases in Scotland is the same as those contracted from water then this can potentially be used in risk categorisation.

5.0 Scoping out microbiological risk assessments for *Salmonella*

Table A.1 Scope out microbiological risk assessments for Salmonella*

Steps	Variables†	Availability of data (Yes/No) and References	Comments
1. Pathogen sources	- prevalence of pathogens in animal sources	cattle (Yes) (Milnes et al. 2008; Kirchner et al. 2012), sheep (Yes) (Milnes et al. 2008) pigs (Yes) (Milnes et al. 2008; Berriman et al. 2013; Powell et al. 2016; Marieret al. 2014), wild animals (No)	- data available at various locations (e.g. abattoir, farm, pen, etc.)
	- concentration of pathogen in faeces	cattle (Yes), (Kirchner et al. 2012) sheep (No), pigs (Yes) (Berriman et al. 2013), wild animals (No)	- data available at various locations (e.g. abattoir, farm, pens, etc.)
2. Transport to PWS and Type of PWS	- transport variables	- See E. coli O157	- information available predominantly for commensal E. coli which can be potentially used as a surrogate for Salmonella.
	- density of animals in the vicinity of PWS	cattle (Yes), sheep (Yes), pigs (Yes), wild animals (No)	- From EDINA Digimap at 2 x2 SqKm level agcensus.edina.ac.uk
	- position of PWS	Yes	-from Local Authorities and DWQR
	- type of PWS	Yes	- DWQR and Local Authority
	- prevalence in PWS	No	NA
	- concentration in PWS	No	NA
3. Survival of pathogens in PWS (or other waters)	- temperature	Yes (Pachepsky et al. 2014)	- only in fresh lake waters
4. Treatment	- proportion of treated PWS	Yes (MacRitchie et al. 2013)	- only for Grampian in 2009 -data needs updated - data needed at national level (Estimate: 80 % of type As; 35 % type B's - personal communication 2019 DWQR)
	- log10 reduction (treatment 1)	Partially (Hijnen et al. 2006)	The only treatment for which there is any reliable information is UV disinfection. The studies cited only consider S. Typhi so may not be representative of other species. Results are variable and studies only tested to 4-log reduction.
	- prevalence before treatment	No	NA
	- prevalence after treatment	No	NA
	- concentration before treatment	No	NA
	- concentration after treatment	No	NA
5. Dose response (DR)	- dose ingested per glass of water	No	NA
	- dose response fitting parameters	Yes (Teunis et al. 2010)	(α, α), Beta –Poisson parameters
	- probability of infection from drinking a glass of water	NA (will be generated on completion of the model) (Teunis et al. 2010)	Beta-Poisson model

*There is no microbial risk assessment model for *Salmonella* infection from drinking water from PWS in Scotland. The main steps and variables needed to develop a model are given.

†The input variables are highlighted in blue text, the validation variables in purple, and the output variables in green. Missing data are marked with "No" red text.

Appendix II. Verocytotoxin-producing *Escherichia coli (VTEC)*

1.0 Hazard identification

1.1 The organism

Verocytotoxin (VT)-producing *Escherichia coli* (VTEC) also known as Shiga toxin-producing *E. coli* (STEC), because of the similarities with *Shigella dysenteriae* (Coia 1998), are zoonotic, gram-negative, rod-shaped gastrointestinal bacterial pathogens (size 0.5 µm by 2µm). They belong to the wide genera of *Escherichia coli,* which naturally inhabit the colon of warm-blooded animals and are the most abundant facultative anaerobe in the human gut (Donnenberg, Whittam 2001, Kaper, Nataro et al. 2004). There have been >380 different VTEC serotypes isolated from humans, the majority of which have also been isolated from animals (Beutin et al. 1998; Karmali et al. 2003). *E. coli* O157:H7 is the most prevalent VTEC strain that causes disease in Scotland (Locking et al. 2001).

1.2 Growth and survival

VTEC pathogens, particularly *E.coli* O157:H7, grow at temperature range of 4 to 44°C, can be resistant to stresses (e.g. acidic conditions – pH 4.1) and can survive for significant periods of time (e.g. 56 days in the environment outside a host), at temperatures as low as 4°C (Conner & Kotrola 1995). They need high a_w to grow (between 0.9 to 0.99) (Clavero &Beuchat 1996), but can survive at a_w as low as 0.34 at both low (5°C) and high temperatures (25°C) (Ryu et al. 1999).

1.3 Inactivation (Critical Control Points and Hurdles)

E. coli O157 has no unusual resistance to heat compared with other micro-organisms. It can be inactivated at 64.3°C in 9.6 seconds in ground beef and at 72°C in 16 seconds during milk pasteurisation (Fernandez 2008). The pathogen is acid tolerant (e.g. survives at pH 4.1) and maximum growth occurs at 44°C and can grow at temperatures as low as 4°C (Buchanan & Bagi 1994; Conner & Kotrola 1995; Fernandez 2008). A 5 log reduction of E. coli O157 is achieved at a concentration of 1.1 mg/L fee (1.2 mg/L total) chlorine in water (Rice et al. 1999). Data for inactivation of *E. coli* O157 by ultraviolet light is presented in Table 8 in the main report.

1.4 Sources

Most members of VTEC are non-pathogenic commensals of the gastrointestinal tract of animals, including farm animals (cattle, sheep, goats, pigs, chickens), pets (cats, dogs) and wild animals (wild birds, rabbits, deer) (Bach et al. 2002; Nataro & Kaper 1998). In Scotland 7.5% (95%CI, 5.4 – 9.6) of faecal samples from beef cattle at slaughter were positive for *E. coli* O157 (Omisakin et al. 2003) and the prevalence was higher during winter (11.2% (95% CI, 8.4-13.9%)) compared with the summer months (7.5% (95%CI, 5.4 – 9.6)) (Ogden et al. 2004). The concentration of pathogen varied between $<10^2$ to 10^6 cfu/g, with 61% of the positive cattle being low shedders ($<10^2$ cfu/g) and 4% high shedders ($>10^4$ cfu/g), respectively (Omisakin et al. 2003). Six and a half percent (6.5%) of Scottish sheep at pasture were positive for *E. coli* O157 (Solecki et al. 2009). It is known that deer can shed VTEC and other animals such as rabbits can also shed these pathogens. However, no data are currently available from the UK on the prevalence and concentrations shed.

2.0 Hazard characterisation: adverse health effects

2.1 Disease symptoms

After a 3-4 day incubation period, VTEC serotypes, such as *E. coli* O157:H7, can cause bloody diarrhoea (97% of cases reported in Europe (ECDC 2007) and 77% in Scotland (Locking et al. 2001). A proportion of cases (2% to 7%), especially in young children, can develop haemolytic uraemic syndrome (HUS), which is a disease characterised by anaemia (caused by destruction of red blood cells), acute kidney failure and low number of thrombocytes in blood. Symptoms of fever and/or vomiting are infrequent (Coia 1998), but approximately half of patients also experience abdominal cramps (Locking, M., Allison et al. 2006). HUS cases may suffer further complications by the development of thrombotic thrombocytopenic purpura (TTP), which is a rare blood disorder that cause blood clots in small blood vessels (Coia 1998).

2.2 Dose response

Several dose response models have been developed for VTEC and in particular for *E. coli* O157 (Haas et al. 2000; Strachan et al. 2005; Teunis et al. 2004; Teunis et al. 2008). These models incorporate outbreak data, but the

model developed by Strachan and colleagues (Strachan et al. 2005) added environmental data for analysis and therefore, at this time, it is probably the most appropriate model to include in microbial risk assessment for PWS. The following best fit parameters (a and b) and corresponding ID_{50} was obtained: a = 5.65×10^{-2} and b = 2.55, ID_{50} = 5.4 x 10^5 (95%CI – 10 to 9x10^{12}) cfu. The 95% ID50 confidence interval is remarkably large, which indicates large variation between strains and/or between human response. If further dose-response data become available, the model can be revised, including the possibility of the variation being better characterised.

2.3 Susceptible population

The highest incidence of VTEC in the UK was observed in children between 1 to 4 years old, indicating that children are likely to have greater susceptibility, but may also have higher exposure (Rotariu et al. 2012; Willshaw et al. 2001). In the USA elderly patients with thrombotic thrombocytopenic purpura (TTP) had clinical and pathologic features similar to patients with HUS, suggesting that these patients are susceptible to *E. coli* O157 infections (Anonymous 1986) . HUS is more common in children and the elderly population (Dundas, Todd et al. 2001) whilst TTP is more common in adults and elderly people (Fernandez 2008). A three year (1987-1989) case-case study in Grampian reported that *E. coli* O157 infections were more common in the agricultural community (MacDonald et al. 1996). Two recent studies in Scotland also suggest that people living in rural areas are more susceptible to the disease than those from urban areas (Innocent et al. 2005; Rotariu et al. 2012). This is likely a consequence of different exposures between the two populations.

2.4 Particular subtypes found in both humans and PWS

E. coli O157 clinical cases reported in Scotland comprised two main phage types PT21/28 (51.9%) and PT32 (15.4%) (Strachan et al. 2015). Shiga toxin *stx2a/stx2c* was present in >60% of cases (Strachan et al. 2015). In an outbreak of *E. coli* O157 in Highlands/Scotland, all isolates collected from cases, water and sheep faeces were phage type 21/28 and identical by pulsed field gel electrophoresis (Licence et al. 2001). Whole Genome Sequencing (WGS) is a relatively recent tool which has been applied to group *E.coli* O157 disease data and identify outbreaks (Dallman et al. 2015). This technique has the potential to be applied for the analysis of *E.coli* O157 isolated from PWS human case isolates with those found in the water. Also, if sufficient numbers of isolates (e.g. tens) become available from PWS these can be compared with those from clinical and other sources.

3.0 Exposure assessment

3.1 Contamination prevalence/ frequency, concentration, survival/ growth in water

In an outbreak investigation of *E.coli* O157 in the Highlands of Scotland phage type 21/28 was isolated from one out of five (1/5) water samples taken from five houses on the same private supply at a campsite (Licence et al. 2001). However, no information on concentration was available.

A survey of PWS in N-E Scotland (Smith-Palmer & Cowden 2010) detected *E. coli* O157 in 1/385 PWS tested. There was no information on the concentration of the pathogen, as the detection was done by enrichment. In the same study (Smith-Palmer & Cowden 2010) the concentration of ordinary *E. coli* in PWS was determined (see Appendix IV on indicator organisms).

A quantitative microbiological risk assessment (QMRA -a mathematical model that predicts risk of illness) for *E.coli* O157 from PWS in Scotland (Rotariu et al. 2012), used the data from Smith-Palmer and colleagues (Smith-Palmer & Cowden 2010). This QMRA modelled the prevalence as a Beta distribution (RiskBeta ([1+1, 385+1]). In the same study (Rotariu et al. 2012) the concentration of commensal *E. coli* in PWS (Smith-Palmer & Cowden 2010) was used as a proxy to estimate the concentration of *E.coli* O157:

$$C_{0157PWS} = C_{E.coliPWS} \times \frac{C_{0157faeces}}{C_{E.colifaeces}} \qquad (6)$$

where $C_{0157faeces}$ and $C_{E.colifaeces}$ were the concentration of bacteria excreted by cattle and sheep in their faeces, in the proximity of PWS by (unpublished data available at University of Aberdeen).

There are no data for VTEC/*E. coli* O157 survival in PWS in Scotland. However, in the USA the survival of a mixture of five nalidixic acid-resistant *E. coli* O157:H7 strains in water (filtered and autoclaved municipal water, reservoir water, recreational lake water) was determined at 8, 15, and 25°C. The initial inoculum was 10^3 cfu/ml. Regardless of the water source, survival was greatest at 8°C (i.e. population decreased by 1 to 2$\log_{10}$ by day 91), whilst at 25°C the pathogen was not detectable (~3$\log_{10}$ decrease) within 49 to 84 days in three of the four water sources. A similar study in Ireland used farm and sterile municipal water (McGee et al. 2002) inoculated with *E. coli* O157. The farm water was inoculated with 10^3 cfu/ml and the sterile water with 10^6 cfu/ml. In the farm water, which was stored outdoors, the pathogen survived for 14 days at ambient temperature (<15°C). Also, in the farm water stored in laboratory conditions at constant temperature (15°C), the pathogen was detected at low

levels (<1 $\log_{10}$ cfu/ml), after 31 days. In the sterile municipal unchlorinated water, a 2.5 $\log_{10}$ reduction was observed in laboratory conditions at constant temperature (15°C), after 31 days, while the organism could not be detected after 17 days in outdoors conditions (<15°C). These studies indicate that specific environmental factors such as temperature have to be considered when taking into account the survival of the pathogens in QMRA modelling. This can be accomplished by fitting survival experimental data for pathogens in water (i.e. determining the coefficients of die-off) at different temperatures and using the fit to estimate the pathogen numbers in water, at each moment after the contamination event. An example of data fitting is given in the next section for *Campylobacter.*

3.2 Dose ingested

The distribution of the daily dose of *E. coli* O157 ingested by the Scottish population that drinks water from PWS, was estimated stochastically in the QMRA by Rotariu and colleagues (Rotariu et al. 2012). This utilised figures on the water consumption distribution in the Scottish populations (see Section 5.3.1 of main report) and the prevalence of *E. coli* O157 and its concentration (see Section 5.4.2.2 of main report). It was assumed 47.5% of Scottish PWS had been treated with 100% efficiency. The percentage of PWS treated was obtained from the telephone survey conducted at Aberdeen University in 2009. No data on treatment efficiency was available from the survey, hence 100% efficiency was considered, which will be an overestimate. The dose from each positive PWS was calculated by multiplying the daily water intake with the concentration of *E. coli* O157 in the water. Figure A.2 shows the dose of *E. coli* O157 ingested for 1000 iterations of the model. The majority of water samples do not contain the pathogen (98.7%). When it is present, the numbers are very low (min=1 cfu, max = 16 cfu, average = 2.8 cfu) (see the inset of Figure A.2). This is in agreement, in terms of dose, with the findings from a later Irish study, which estimated that 95% of the people consuming water from groundwater private sources ingest VTEC doses ranging between 1 to 15 cfu daily (Hynds et al. 2014).

4.0 Risk characterisation

4.1 Incidence

One of the earliest surveillance studies of VTEC in Scotland was based on data obtained between 1984 to 2004 (Locking, M., Allison et al. 2006). These data showed a steady increase of the incidence of *E. coli* O157 until 1996 (10 cases/100,000 population), followed by a decline until 2004 (4.1 cases/100,000 population). Over the 10 years (2005-2014) the incidence of *E. coli* O157 in Scotland has been relatively constant (~4 cases/100,000) (Locking, et al. 2014). During 2000-8 approximately 50% of the *E. coli* O157 cases belonged to phage type PT21/28 (Locking & Cowden 2009), which was also the commonest phage type (i.e. accounted for 42% of the cases) in the period between 2009 to 2013) (Locking et al. 2014).

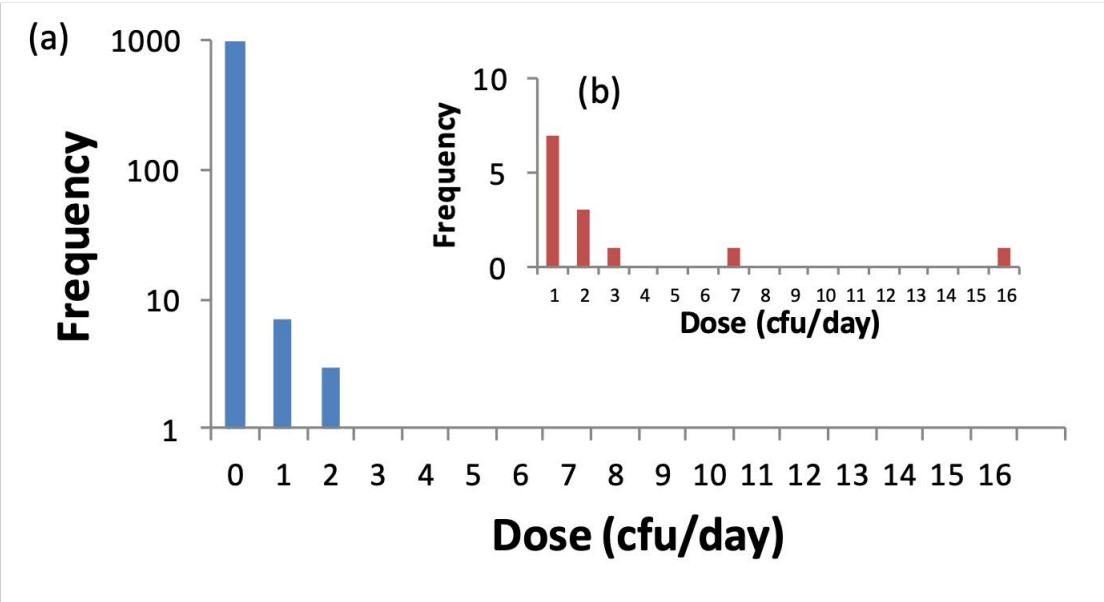

Figure A.2 Distribution of the daily dose of E.coli O157 in the Scottish population: (a) overall distribution including water samples clear of pathogens; (b) distribution of positive samples (excluding zero concentration).

4.2 Clinical consequences of infection

Between 30 and 57% of *E. coli* O157 and other VTEC cases require hospital treatment (Locking et al. 2006; Locking et al. 2001; Park et al. 1999). In Scotland 43% (90/210) of reported cases were hospitalised in 2004 (Locking et al. 2006). The overall mortality rate of *E. coli* O157 is a small proportion of cases and has been reported to be approximately 3% in USA and Europe (Coia 1998). The mortality rate of HUS has been reported to be 2.5% in the UK (1997-2001) and 3-5% elsewhere in Europe (ECDC 2007; Lynn et al. 2005). Karmali *et al.* (1985) reported that 8-10% of *E. coli* O157:H7 infections develop into renal complications (ECDC 2007; Locking et al. 2006) that are characterised by potentially fatal acute renal failure, thrombocytopenia and microangiopathic haemolytic anaemia (Coia 1998; ECDC 2007) In Scotland, 82.2% of all HUS cases (n=73, 2003-2005) were significantly associated ($P<0.001$) with prior *E. coli* O157:H7 infection (Pollock et al. 2006). Over half (55%) of Scottish *E. coli* O157 associated HUS cases occurred in children < 5 years old (Lynn et al. 2005). HUS adult cases may suffer further complications which involve the nervous system and other organs. These complications may manifest as thrombotic thrombocytopaenic purpura (Coia 1998). In both HUS and TTP, organs including the brain, myocardium and pancreas may be affected, with consequent development of encephalopathy, cardiomyopathy and diabetes mellitus (Coia 1998).

4.3 Outbreaks

During the 10 years (2005 to 2014) there were 83 *VTEC* outbreaks reported in Scotland (Anonymous, 2016). These outbreaks comprised 466 cases (~5.6 cases/outbreak). For 7 of these water was the suspected vehicle and the location was either private house or holiday accommodation. However, for 6/7 outbreaks the source of water (e.g. PWS) was not specified. The 7th outbreak was caused by *E. coli* O157, phage type PT21/28, occurred in holiday accommodation, water from a PWS was the probable vehicle, and 10/15 cases were confirmed positive for *E. coli* O157 (Smith-Palmer & Cowden 2010).

During 1990 to 2014 there were 4 outbreaks of *E. coli* O157:H7 in Scotland, which were reported in the scientific literature and were related to drinking water, including from PWS (Saxena et al. 2015). Two of these outbreaks were related to PWS. In the first PWS Scottish outbreak four primary cases occurred simultaneously in the town of Tarves (N-E Scotland) and the water from the PWS of patients was heavily contaminated with faecal *E. coli*, but *E. coli* O157 was not isolated (Dev et al. 1991; MacDonald et al. 1996). In the second PWS Scottish outbreak, that took place in 1999, all isolates from cases, water and sheep faeces were confirmed as phage type 21/28 and identical by pulsed field gel electrophoresis, providing evidence that the outbreak was PWS related (Licence et al. 2001). Three other waterborne PWS outbreaks have been reported from the rest of the UK (Saxena et al. 2015).

4.4 Sporadic cases

Despite the majority of *E.coli* O157 cases in Scotland being sporadic (e.g. 81% (n = 210) were sporadic in 2004) (Locking et al. 2006), there are no reports in the scientific literature on sporadic cases of *E.coli* O157 associated with untreated water (most likely originating from PWS). A case-control study of sporadically acquired *E. coli* O157 in Scotland (1996-1999) found a negative association between cases and consumption of bottled water (Locking et al. 2001). In the same study, the consumption of domestic tap water (unheated) and drinking untreated water were not associated with disease, but there was no information available on drinking from a PWS (Locking et al. 2001). There are no other case-control studies in the UK which have investigated PWS as a potential source of *E. coli* O157. However, in a nationwide case-control study in the USA, drinking unchlorinated water was associated with *E. coli* O157 disease (OR 2.4; 95% CI, 1.1-5.7)) (Slutsker et al. 1998). Further, as mentioned above (6.2.4) there are Scottish outbreaks which are associated with PWS. These outbreaks and the results of the USA study suggest that there might be a risk of acquiring sporadic *E. coli* O157 illness from PWS in Scotland and this needs to be investigated. However, definitively linking of sporadic cases of *E. coli* O157 with PWS is difficult to ascertain directly, since PWS are not sampled when sporadic cases occur. Therefore, there is a need to ask *E.coli* O157 cases for consumption of water from PWS and compare these with matched controls (e.g. controls from the same geographical area). As the number of sporadic *E.coli* O157 cases in Scotland is relatively small, conducting UK wide case-control studies will be a better option to obtain statistically credible results.

4.5 Risk assessments

QMRA of PWS in Scotland

A quantitative microbial risk assessment (QMRA) was developed in Scotland to estimate the probability of illness and the number of cases of *E. coli* O157 from drinking water from PWS (Rotariu et al. 2012). The model used an exposure assessment conducted at the University of Aberdeen to estimate the water intake of people living on PWS (MacRitchie et al. 2013). The concentration of the pathogen was estimated using the concentration of ordinary *E.coli* in PWS as a proxy and the amount of *E. coli* and *E. coli* O157 excreted by animals in the proximity

of PWS (see 5.4.2.2) and the number of exposures (34 million glasses drunk /year) was determined as described in 5.4.1.1. The probability of infection per glass of water was modelled using a Beta-Binomial function,

$$P_{inf} = 1 - (1 - P_{I\_1})^{Dose} \quad (9)$$

where $Dose = V_{GlassWater} \times Concentration$.

This was applied to estimate the exposure of Scottish population (34 mil glasses of water consumed in one year). It was estimated that approximately 10% of *E. coli* O157 cases were due to private water supplies the remainder from other sources (e.g. food, exposure from environment) (Rotariu et al. 2012)).

Quantitative risk factor approach (QRFA) to estimate the relative importance of pathways of infection with E. coli O157

A QRFA using data at postal district level was developed in Scotland to estimate the relative risk of becoming infected with *E. coli* O157 from food sources, environment and PWS (Rotariu et al. 2012).

A waterborne (W) proxy risk was estimated as:

$$W \quad N_{PWS} \times N_{E.coli0157} \quad (10)$$

where N_{PWS} were the number of properties on PWS in Scotland for each postal district and $N_{E.coli0157}$ was the total number of *E. coli* O157 excreted by cattle and sheep (calculated by estimating the average number of *E. coli* O157 excreted per gram of faeces multiplied by the average weight of faeces excreted per animal per day, multiplied by the number of animals in the postal sector – this was done separately for cattle and sheep and the results were summed).

A food (F) proxy risk was estimated as:

$$F \quad P \quad (11)$$

where P was the population in each postal district in Scotland.

An environment (E) proxy risk was estimated as:

$$E \quad P \times N_{E.coli0157} \quad (12)$$

The regression model combined the three risks mentioned above as following:

$$N_{cases} \quad aF + bE + cW \quad (13)$$

where a, b, c were regression coefficients. The regression was performed over all Scottish postal districts (n=38), the regression coefficients were determined, and the cases and relative risks from each factor (food, environment and water) were estimated.

4.6 Qualitative/Quantitative estimate of risk from the risk assessments

QMRA of PWS in Scotland

The average daily probability of illness from drinking Scottish PWS water contaminated with *E. coli* O157 was estimated to be 1.31×10^{-5}(min =0, max =1) (Rotariu et al. 2012). The distribution of the probability of illness shows that 99.97% of PWS consumers are not exposed (black column in Figure A.3).The estimated number of cases was 162 (95%CI 144–180) per year, based on 34 million glasses drunk from PWS. This is an overestimate since there are only approximately 300 cases (including outbreaks) of O157 reported per year in Scotland.

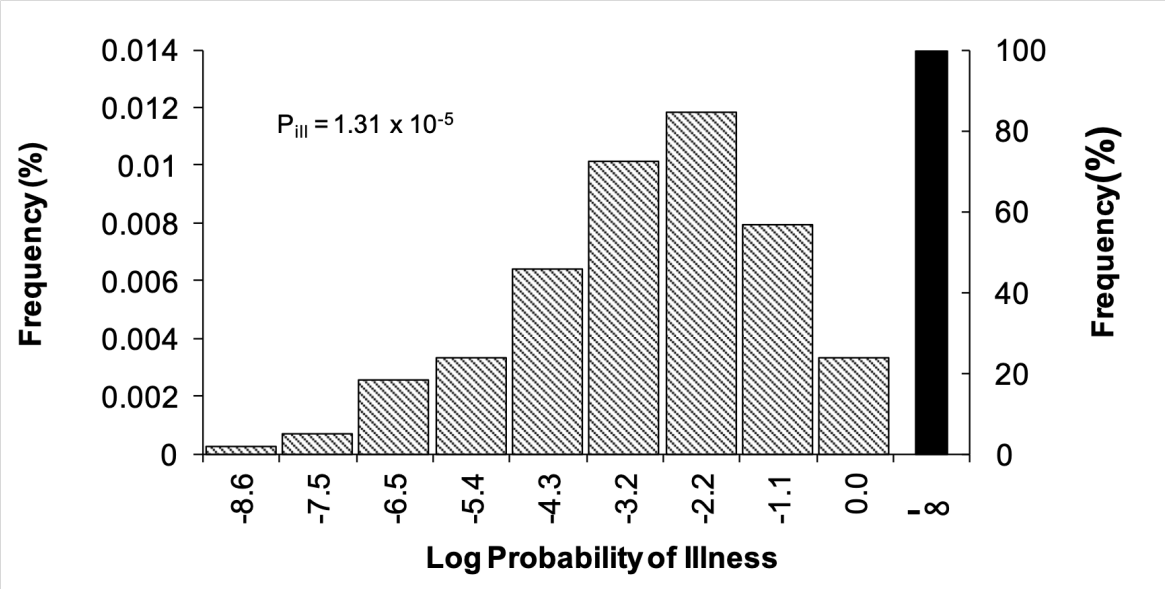

Figure A.3 The distribution of probability of illness from drinking E. coli O157 contaminated PWS in Scotland.

Quantitative risk factor approach (QRFA) to estimate the relative importance of pathways of infection with E. coli O157

The quantitative risk factor approach for *E.coli* O157 developed in Scotland found that water was associated with only a small proportion of cases – 7.3% (CI 0-16%) (Rotariu et al. 2012).

4.7 Risk categorisation

The rationale for categorisation of PWS/hazard combinations is presented in Appendix VI.

Disease incidence

The QMRA model, developed by pathway of transmission (water, food and environment), found the risk of disease from PWS as being the least contributor (9.9%) to the total burden of *E. coli* O157 infection (Rotariu et al. 2012). The other contributing factors were the environment (e.g. pastures grazed by cattle and sheep) (34%) and food (e.g. burgers) (56.1%). The QRFA model ranked the risk from PWS as being 7.3% of the total burden of *E. coli* O157 infection (Rotariu et al. 2012). The other contributing factors were the food (26.9) and environment (65.8%).

The burden of disease caused by drinking water from private water supplies is unknown. Therefore, the overall incidence, from all sources, for each pathogen is used as an alternative way for risk categorisation (see Table A1 in Appendix VI). *VTEC, Shigella and Giardia* were categorised as lowest risks (rank 5) in terms of incidence.

Disease severity

The severity of disease from waterborne infections is also unknown. This is also required for risk categorisation but has not been done here. However, if it is assumed that the spectrum of disease obtained from all cases in Scotland is the same as those contracted from water then this can potentially be used in risk categorisation.

5.0 Scoping out microbiological risk assessments for Verocytotoxin-producing *Escherichia coli (VTEC)*

Table A.2 Scope out microbiological risk assessments for *E. coli* O157**

Steps	Variables†	Availability of data (Yes/No) and References	Observations
1. Pathogen sources	- prevalence of pathogens in sources	cattle (Yes) (Omisakin et al. 2003; Ogden et al. 2004; Solecki 2008)	- surveys at University of Aberdeen - data available at farm level for cattle and sheep in both herds/flocks and individual animals, respectively
		Sheep (Yes) (Solecki et al. 2009; Ogdenet al. 2005)	
		wild animals (No)	
	- concentration of pathogen in faeces	cattle (Yes) (Omisakin et al. 2003; Ogden et al. 2004; Solecki 2008),	- surveys at University of Aberdeen for individual animals at farm level
		sheep (Yes) (Soleckiet al. 2009; Ogden et al. 2005),	
		wild animals (No)	

Steps	Variables†	Availability of data (Yes/No) and References	Observations
2. Transport to PWS and Type of PWS	- transport variables	- Generic rainfall detachment: (Blaustein et al. 2015b; Muirhead et al. 2006; Brennan et al. 2012; Blaustein et al. 2015a) - Leaching / release in overland flow: - Cattle (Yes) (Muirhead et al. 2006; Muirhead et al. 2005) - Sheep (Yes) (Moriarty & Gilpin 2014) - Deer (Yes) (Guber et al. 2015) - Other wild animals (No) - Generic overland flow transport: (Tyrrel & Quinton 2003; Collins et al. 2005; Blaustein et al. 2015b; Muirhead et al. 2006; Muirhead et al. 2005) - Drainage / subsurface transport - Generic (Brennans et al. 2012: Artz et al. 2005) - Cattle (Yes) (Oliver et al. 2005) - Sheep (Yes) (Vinten et al. 2004) - Wild animals (No) - Generic macropore sub-surface transport (Martins et al. 2013: Arnaud, Best et al. 2015) - Generic stream sediment deposition / entrainment: (Pachepsky, Shelton 2011: Pandey et al. 2012)	- Models of *E. coli* transport typically assume a sediment-transport style involving processes of release / detachment, transport and deposition. - Most cited studies here use generic *E. coli* - would need to assume *E. coli* O157 experiences same transport processes. - Generic studies provide models that can be applied to multiple FIOs or consider behaviour of *E. coli* (O157) independent of animal source.
	- density of animals in the vicinity of PWS	cattle (Yes), sheep (Yes), wild animals (No)	- From EDINA Digimap at 2 x2 SqKm level agcensus.edina.ac.uk
	- position of PWS	Yes	-from Local Authorities
	- type of PWS	Yes	- DWQR and Local Authority
	- prevalence in PWS	Yes (Smith-Palmer & Cowden 2010)	-survey in N-E Scotland
	- concentration in PWS	No	- calculated (Rotariu, Ogden et al. 2012) but needs updated; not obtained from a transport model

Steps	Variables†	Availability of data (Yes/No) and References	Observations
3. Survival of pathogens in PWS (or other waters)	- temperature	Yes (McGee et al. 2002)	- farm and sterile municipal water
4. Treatment	- proportion of treated PWS	Yes (MacRitchie et al. 2013)	- only for Grampian in 2009 -data needs updated - data needed at national level (Estimate: 80 % of type As; 35 % type B's - personal communication 2019 DWQR)
	- log_{10} reduction (treatment 1)	Partially (Hijnen et al. 2006: Chen et al. 2009)	The only treatment for which there is any reliable information is UV disinfection. Unlikely to represent all species. 4-log reduction with 10 mJ/cm^2 UV.
	- log_{10} reduction (treatment 2	(Petterson & Stenstroem 2015; Hokajarvi et al. 2018)	0.04-5.2 log reduction (will vary depending on water quality and chlorine dose. NB usually either chlorine or UV used. Data are generic *E. coli*
	- log_{10} reduction (treatment 3	(Abbaszadegan et al. 1997)	6-log reduction in *E. coli* O157 with a point of use activated carbon plus UV filter (UV fluence not given).
	- prevalence before treatment	No	NA
	- prevalence after treatment	No	NA
	- concentration before treatment	No	NA
	- concentration after treatment	No	NA
5. Dose response (DR)	- dose ingested per glass of water	No	- calculated (Rotariu, Ogden et al. 2012) but will need re-evaluated
	- dose response fitting parameters	Yes (Strachan et al. 2005)	(α, β), Beta –Poisson parameters
	- probability of infection from drinking a glass of water	NA (will be generated on completion of the model)	Beta-Poisson model (Generated in the model developed by Rotariu and colleagues, (Rotariu, Ogden et al. 2012) and needs re-evaluated)

**There is a microbiological risk assessment model for *E. coli* O157 infection from drinking water from PWS in Scotland (Rotariu et al. 2012). This needs to be updated to include transport and treatment as well as incorporation of other missing data that are detailed above.

†The input variables are highlighted in blue text, the validation variables in purple, and the output variables in green. Missing data are marked with "No" red text.

Appendix III. *Campylobacter*

1.0 Hazard identification

1.1 The organism

Campylobacter are zoonotic, small (0.2–0.8µm×0.5–5µm) gram-negative, slender, spirally curved rod-like bacteria which cause gastroenteritis in humans (Silva et al. 2011). The genus *Campylobacter* comprises over 30 species, with *C. jejuni and C. coli* being the main two species causing gastroenteritis in Scotland (Sheppard et al. 2009) and in the developed world (Park, 2002).

1.2 Growth and survival

Campylobacter species are able to grow between 37 and 42˚C, but incapable of growth below 30˚C, with an optimum temperature of 41.5˚C (Silva et al. 2011). However, the pathogen can survive for >20 days at low temperatures (e.g. as low as 4°C) in a range of environmental matrices (soil, faeces, water– unpublished data). It does not grow outwith an animal host, but can persist in the environment (e.g. for 20 days in poultry litter and for 60 days in well water at 4°C) (Gonzalez & Hanninen 2012; Kassem et al. 2010). The pathogen does not grow at a_w <0.987 and optimal growth occurs at a_w =0.997 (Bull et al. 2006). *Campylobacter* does not survive in pH<4.9 and >9.0 and grows optimally between pH 6.5-7.5 (Silva et al. 2011).

1.3 Inactivation (Critical Control Points and Hurdles)

Campylobacter can be inactivated by heat and has a small decimal reduction time (*D*-value - the time to be reduced by one $\log_{10}$) (e.g. *D* = 5.3 minutes in brain heart infusion broth at 55°C) (Silva et al. 2011). In diluents (e.g. 10% dimethyl sulfoxide or 10% glycerol) temperatures <-15°C inactivates, but does not kill the pathogen (i.e. it can be recovered), in less than 3 days (Stern & Kotula 1982). Inactivation by ultraviolet light is presented in Table 8 in the main report. A concentration of 0.1 mg of free chlorine per litre was found to provide >99% inactivation in water (Blaser et al. 1986).

1.4 Sources

Campylobacter are carried asymptomatically in the gut of many warm-blooded wild, domestic, and farm animals (Moore et al. 2005; Wassenaar & Blaser 1999). Reservoirs include cattle, sheep (Stanley & Jones 2003a), poultry (Bull et al. 2006), wild birds (Griekspoor et al. 2013) and pigs (Boes et al. 2005), and they harbour high levels of strain diversity (Colles et al. 2003). Companion animals, including cats and dogs, also carry *Campylobacter* (Workman et al. 2005; Lee et al. 2004). *Campylobacter* excreted from these reservoirs can be found throughout the environment including soil (Santamaria & Toranzos 2003), beach sand (Bolton et al. 1999), sewage (Jones 2001), and groundwater (Stanley et al. 1998). The prevalence and the concentration of *Campylobacter* in faeces of a wide variety of animal sources from Scotland, including cattle, sheep, pigs, a range of wild and domesticated avian species and pets have been published (Ogden et al. 2009).

2.0 Hazard characterisation: adverse health effects

2.1 Disease symptoms

Campylobacteriosis develops usually within 2-3 days following ingestion of this organism and the most frequent symptom is diarrhoea (limited/ voluminous stools, watery or bloody) (Moore et al. 2005) . Severe abdominal cramps is also a common symptom, whilst vomiting is less frequent (Smith-Palmer & Cowden 2010). In up to two-thirds of cases musculoskeletal, joint swelling, or sensory problems and numbness are reported (Zia et al. 2003). Additionally, *Campylobacter jejuni* contribute to 14% of all cases of Guillain-Barré syndrome (GBS) in the UK (a neuropathic condition characterised by an ascending flaccid paralysis from the legs to upper body) (Tam et al. 2006). Other symptoms (e.g. fever, headache, asthenia, and anorexia) may precede diarrhoea (Moore et al. 2005). Longer term colitis which sometimes resemble the symptoms of irritable bowel syndrome (IBS) may also occur. In extreme cases death may also occur and in the UK, *Campylobacter* causes more than 100 deaths each year.

2.2 Dose response

A number of dose response models have been developed for campylobacteriosis (Medema et al. 1996; Teunis et al. 1999b; Teunis et al. 2005; Teunis & Havelaar 2000). These studies used dose-response data from human outbreaks (Evans et al. 1996) and/or feeding studies on adult volunteers (Black et al. 1988). The last study by Teunis and

colleagues (Teunis et al. 2005) used the information from an outbreak in children which consumed milk at a farm visit. Using the exponential model, the best fit parameter was r = 0.118, resulting in an ID_{50} = 5.8 cfu (95%CI – 1 to 9 cfu).

2.3 Susceptible population

The aetiology of campylobacteriosis is complex but empirical and analytical epidemiology found that different age groups have different disease incidences (Strachan et al. 2013; Strachan et al. 2009). Hence, children living in rural areas have higher disease incidence than those from urban areas, which may be due to different exposure to environmental factors. Also, increases in disease rates was observed in the last decade in the elderly population. Cohort, case-control and empirical epidemiology studies show that people taking proton pump inhibitor (PPI) medication are at higher risk of becoming ill (Brophy al. 2013; Mughini Gras et al. 2012; Strachan et al. 2013). However, it is possible that usage of PPI's may not be causal, but an indicator of an underlying health problem that leads to greater susceptibility of *Campylobacter* infection.

2.4 Particular subtypes found in both humans and PWS

A case-control study in N-E Scotland found drinking from PWS as a risk factor for contacting campylobacteriosis (odds ratios - 3.062 (2.056 – 4.562)) (Smith-Palmer & Cowden 2010). However, in the same study PWS water testing found no association between *Campylobacter* isolates (genotyped by multi locus sequence typing (MLST)) from cases and isolates from PWS (Smith-Palmer & Cowden 2010). This was due to the small number (2/77) of PWS samples that tested positive for *Campylobacter* and was probably due to the delay in water sampling after the human disease event. A similar study, which analysed the causes of a waterborne *Campylobacter* outbreak from a Greek island, revealed a strong epidemiological association between disease and tap water consumption from a rural source (Karagiannis et al. 2010). However, no *Campylobacter* was isolated in the tested water. This is expected as *Campylobacter* dies-off rapidly (e.g. 1-2 $\log_{10}$ cfu/ day - see subsection 5.4.2.3 in the main report) and there would need to have been repeated contamination to enable detection of this pathogen. In Finland, clustering using Bayesian Analysis Population Structure (BAPS) found a significant association ($P < 0.0001$) between human isolates and isolates from environmental waters (de Haan et al. 2013). A similar result was found in Canada where similar Clonal Complexes (CC 21, CC45 & CC61) were found both in river/tributary water samples and human faecal samples.

These studies provide some evidence that campylobacteriosis can be contracted from contaminated water sources (including PWS), but more data are required to test a putative association of the disease with PWS. This would need intensive sampling of PWS concurrently with episodes of human infection, genotyping of the isolates and their comparison with isolates from human samples.

3.0 Exposure assessment

3.1 Contamination prevalence/ frequency, concentration, survival/ growth in water

In a survey of drinking water sources (PWS and public) in NE Scotland (Smith-Palmer & Cowden 2010) 2.6% (2/77) of the PWS sources and none of public mains supplies (0/925) were positive for *Campylobacter* (Smith-Palmer & Cowden 2010). Four of the water sources were a combination of private and public supplies and one of these (25%) was positive for *Campylobacter*. *Campylobacter* was detected after enrichment and therefore enumeration was not carried out and so no counts were available. Also, no survival analysis was carried out in this study (Smith-Palmer & Cowden 2010). In Cumbria (1994) groundwater originating from a spring was contaminated with *Campylobacter* from a dairy farm (Stanley & Jones 2003b). There was no information on the concentration of the pathogen.

Unpublished data from the research group at University of Aberdeen showed that die-off (decay) rates of *Campylobacter* are different at different temperatures, in sterile water (Figure A.4). The die-off is faster at higher temperatures (e.g. 1 to 2 $\log_{10}$ cfu/day at 15°C) than at lower temperatures (e.g. 0.5 to 1 $\log_{10}$ cfu/day at 4°C). This suggests that in those PWS where the temperature is below 10°C (e.g. a well), the pathogen will survive for longer times than in those where the temperature is higher (e.g. 10°C to 15°C in surface water during the summer) (Gavriel et al. 1998). These data also show a differential survival by genotype (e.g. MLST genotype ST21 originating from a cow survives longer than the other strains). These experiments were conducted in sterile water and that is unlikely to be the case in PWS as there may be traces of organic matter as well as other organisms present which may affect the survival/growth rates.

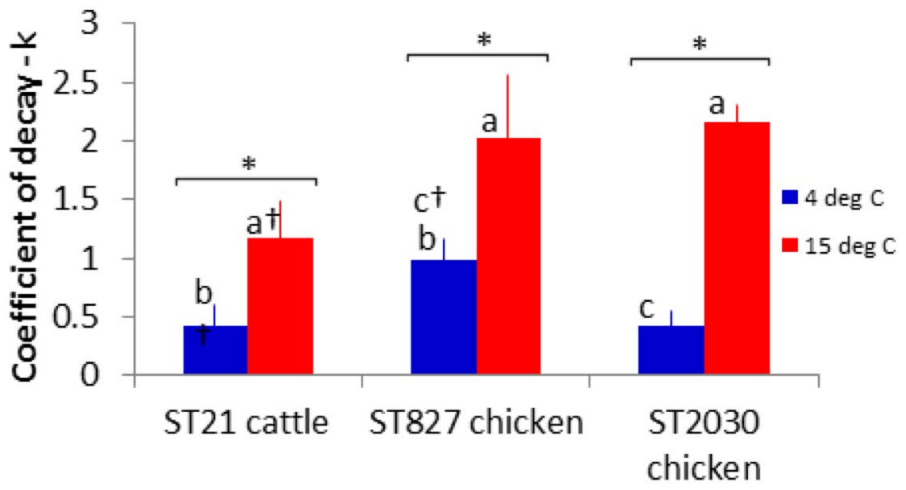

Figure A.4 Die-off (decay) coefficients of different strains of Campylobacter in sterile water (Note: the die-off coefficient, k, gives the number of $\log_{10}$ reduction/day of the pathogen numbers).

3.2 Dose ingested

There is no information on the dose of *Campylobacter* ingested from drinking water from PWS in Scotland or the UK.

4.0 Risk characterisation

4.1 Incidence – Scotland

Reported campylobacteriosis data from Scotland showed that the incidence doubled between 1990 (59.7 cases/100,000 population) and 2014 (124 cases/100,000 population) (Strachan et al. 2013). The temporal pattern of human campylobacteriosis in Scotland follows the same trend as reported in England and Wales. There was a maximum incidence in 2000 (128 cases/ cases/100,000 population), followed by a decrease until 2004 (86 cases/100,000 population) and rose again until 2014 (124 cases/100,000 population) (Strachan et al. 2013).

4.2 Clinical consequences of infection

Up to 65% of *Campylobacter* cases develop musculoskeletal, joint swelling, or sensory problems (Zia et al. 2003). Approximately 10% of individuals reported as having campylobacteriosis are hospitalised (Zia et al. 2003). In Scotland, between 1997 to 2012, the average percentage of hospitalised cases was 8.1% (range 6.3% to 11.6%) and was highest (17%) (range: 12.7% - 22%) in the elderly (Strachan et al. 2013). The average hospitalisation rate was 8.9 hospital discharges/100,000 population (range: 5.9/100,000 – 14/100,000) and was also highest (17.8/100,000, (range: 8.8/100,000 – 34/100,000)) in the elderly (Strachan et al. 2013). In this group the hospitalisation rate doubled (from 16.7/100,000 to 33.8/100,000) between 2007 – 2012 (Strachan et al. 2013).

Severe sequelae occur with approximately 1 in 1000 *Campylobacter* reported infections which develop in Guillian-Barré syndrome (Allos & Blaser 1995: Altekruse et al. 1999) with a mortality rate of 5% (Altekruse et al. 1999). Seventy percent of GBS cases make a full recovery (Blaser 1997). In England 14% of GBS cases could be attributable to symptomatic *C. jejuni* (Tam et al. 2006).

4.3 Outbreaks

During the 10 years of 2005 to 2014 there were 13 *Campylobacter* outbreaks in Scotland (Anonymous, 2016a). These outbreaks accounted for approximately 260 cases (~20 cases/outbreak) which is approximately 0.45% of reported cases. MLST analysis of sporadic cases revealed that approximately 3.2% of cases are associated with small household outbreaks (Rotariu et al. 2010).

In Scotland there was a single mixed *Campylobacter & Cryprosporidium* outbreak related to water, consumed at a hospital (Smith-Palmer & Cowden 2010). There was no further information on the type of water consumed. In England a mixed *Campylobacter/Cryptosporidium* outbreak was associated with an untreated PWS in the early 90's (Duke et al. 1996). Forty-three out of 200 residents (22%) connected to the PWS had disease symptoms. Fifty-five percent (11/20) of the stool specimens from cases contained pathogens (4 *Campylobacter*, 5 *Cryptosporidium* and 2 both). Microbiological analysis of water samples revealed no

Campylobacter or *Cryptosporidium* contamination, but high levels of commensal *E. coli* (up to 112/100 ml) were detected. Three dead lambs were found in a collecting chamber supplying the main storage chamber which may have contributed to the contamination.

4.4 Sporadic cases

The vast majority of *Campylobacter* cases are sporadic (Pebody et al. 1997; Rotariu et al. 2010). In Scotland private water supplies were associated with sporadic cases of *Campylobacter* infection in a case-control study conducted between August 2005 and November 2007 (Smith-Palmer & Cowden 2010). The adjusted odds ratio for the risk factor of being on a PWS was 3.062 (2.056 – 4.562), for all cases resident in Aberdeen city and Aberdeenshire compared to controls (Smith-Palmer & Cowden 2010). In the same study water tests were performed trying to identify *Campylobacter* strains in both humans and PWS, but *Campylobacter* was only found in 2/77 PWS samples from properties which reported campylobacteriosis. The microbiological sampling would have taken place sometime (1-2 weeks) after the case had contacted the disease. In an English case-control study (Evans et al. 2003) consuming bottled water was positively associated with campylobacteriosis. This was opposite to the finding by Locking et al. (Locking et al. 2001) which found a negative association between cases of *E. coli* O157 and drinking bottled water. Evans and colleagues (Evans et al. 2003) do not specify if the bottled water had been tested for microbiological quality. The authors specified that indicator organisms (e.g. coliforms) were reported by previous studies as being found in mineral water, particularly in uncarbonated water supplied in plastic bottles or bottled by hand (Hunter 1993). Hence, the potential for it to be a source of *Campylobacter* (Evans et al. 2003).

4.5 Risk assessments

There is no risk assessment for *Campylobacter* and drinking water from PWS in Scotland. However, several case-control (Roux et al. 2013, Smith-Palmer & Cowden 2010) and case-case (Roux et al. 2013; Bessell et al. 2012; Mughini Gras et al. 2012) studies, based on Scottish or European data, supply useful information which may contribute to the understanding of the importance of PWS to the aetiology of campylobacteriosis. The potential utility offered by these studies is summarised below.

Case-control in N-E Scotland

A case-control study was conducted in NE Scotland between August 2007 to November 2007 (Smith-Palmer & Cowden 2010). A variety of risk factors including having a property being supplied by a PWS source were considered. Eight percent (63) of cases and 2.8% (45) of controls had a private water supply. Population attributable fraction (PAF) for this risk factor can potentially be determined (Rockhill et al. 1998).

Models using genetic information

a) Case-case model on environmental attributed Campylobacter cases

A combined case-case regression/genetic attribution model was developed in Netherlands (Mughini Gras et al. 2012). The model considered environmental attributed cases (water, sand and wild birds) as a subset of the total cases which were used for regression analysis. Non-food specific risk factors (e.g. swimming, ownership of pets, contact with animals and use of medicine) were considered in this model and population attributable risks (PAR) and corresponding P-values for significance analysis were determined. Exposure to drinking water from PWS was not considered in this model, probably due to the lack of information on exposure. Repeating the model by Gras and colleagues (Mughini Gras et al. 2012) for environmental attributed Scottish cases and exposure conditions (including to PWS) specific to the Scottish population, would allow determination of the PAR for exposure to PWS.

b) Case-case model on chicken and ruminant attributed Campylobacter jejuni cases

Another case-case regression/genetic attribution model was developed in Scotland (Bessell et al. 2012). The *Campylobacter jejuni* human cases were assigned by attribution (using STRUCTURE software (Pritchard et al. 2000)) into chicken and ruminant sources. Then a mixed case-case logistic regression analysis was used to determine the importance of a range of risk factors (gender, age, population density, socio-economic status, geographic coordinates, season, density of farmed animals, travel) for being infected with *C. jejuni* from these two sources. Odds ratios (OR) and confidence intervals (CIs) were used to quantify the difference by source. PWS were not considered as a risk factor in this model but could be incorporated to ascertain their importance in contracting campylobacteriosis.

c) Case-control and case-case studies on Campylobacter jejuni and Campylobacter coli

Three case-control/case-case studies explored the aetiology of *C. coli*/*C. jejuni* over one year (2005-2006) across Scotland (Roux et al. 2013). In the first study 307 *C. coli* clinical cases were compared with 921 controls generated by randomly sampling the human population. In the 2nd study 307 *C. coli* cases were compared with 2,733 *C. jejuni* cases as controls. In the 3rd study 113 *C. coli* cases attributed to chicken were compared with the 181 non chicken cases as controls. In all these studies mentioned above, multivariate logistic regression was

employed to determine risks factors associated with the disease. Odds ratio and confidence intervals were used to quantify the differences between groups. As in the (b) above no information on PWS was taken included. However, it could be and again would provide another way of estimating the importance of PWS in being a source of human campylobacteriosis.

4.6 Qualitative/Quantitative estimate of risk from the risk assessments

Case-control in NE Scotland

Living on a property supplied by a PWS was found to be a risk factor in the study by Smith-Palmer and Cowden (2010). The associated PAF was 8.2%, which means that 8.2% of *Campylobacter* cases could have been prevented following the elimination of exposures to untreated PWS. Some other factors such as drinking from a drinking dispenser, fountain or a river and practicing water sports (swimming, canoeing, sailing, and surfing) were not significant for campylobacteriosis. This case-control study emphasizes the importance of PWS as a putative risk factor for *Campylobacter* disease. However, the study was performed only in N-E Scotland and over a short timescale (two years), resulting in only 63(8%) cases and 45(2.8%) of controls drinking from a PWS. Therefore to (in)validate its findings, it is desirable to repeat the study at a national scale and over a longer time scale.

Models using genetic information

a) Case-case model on environmental attributed Campylobacter cases

The Dutch case-case study (Mughini Gras et al. 2012) offers a good example on how information about risk factors, combined with genetic information about *Campylobacter* strains from cases and sources of origin, can be utilised to quantify the relative importance of these factors in terms of disease aetiology. The study found domestic swimming in a pool as an environmental risk factor associated with campylobacteriosis (PAR 28% (2-64%)). Other factors such as contact with pets/farm animals (0.4% (0.2–1.0%)), ownership of dogs (3.5% (1.0–12.0%)), having a chronic gastrointestinal (GI) disease (35% (6–63%)), contact with people having a GI condition (3.4% (1.3–8.7%)), were also found as risk factors significantly associated with campylobacteriosis of environmental origin.

If information about exposure to risk factors (including exposure to PWS) for Scottish human *Campylobacter* cases was available, and the pathogenic isolates (both from cases and sources) were collected and genotyped, then the development of a similar model (Mughini Gras et al. 2012) would be possible. This would establish whether drinking water from PWS is an important risk factor for human campylobacteriosis and if so, the PAR could then be determined.

b) Case-case model on chicken and ruminant attributed Campylobacter jejuni cases

In the regression/genetic attribution model for *C. jejuni* human cases (Bessell et al. 2012), a total of 1,599 (46.3%) cases were assigned to poultry, 1,070 (31.0%) to ruminant and 67 (1.9%) to wild bird sources; the remaining 715 (20.7%) did not have a source that could be assigned with a probability of greater than 0.95. Compared to ruminant sources, cases attributed to poultry sources were typically among adults (odds ratio (OR) = 1.497, 95% confidence intervals (CIs) = 1.211, 1.852), not among males (OR = 0.834, 95% CIs = 0.712, 0.977), in areas with population density of greater than 500 people/km^2 (OR = 1.213, 95% CIs = 1.030, 1.431), reported in the winter (OR = 1.272, 95% CIs = 1.067, 1.517) and had undertaken recent overseas travel (OR = 1.618, 95% CIs = 1.056, 2.481). This demonstrates that human *C. jejuni* infections that are attributable to sources in ruminants, occur more in summer, in rural areas, are less travel related and are more frequent in children than those assigned to poultry sources. The rural "fingerprint" of these ruminant attributed cases has the possibility that exposure to water from PWS may have a contribution to the incidence of disease. To confirm this, it is recommended that future case-case studies on *Campylobacter* should collect and use information on exposure to environmental factors (including drinking water from PWS) and quantify their relative importance.

c) Case-control and case-control studies on Campylobacter jejuni and Campylobacter coli

The case-control study (1st study) performed by Roux et al. (Roux et al. 2013) found an increased risk of *C. coli* infection in people older than 19 years (OR = 3.352), and during the summer months (OR = 2.596), while residing in an urban area decreased the risk (OR = 0.546). The 2nd study by Roux et al. (Roux et al. 2013) also showed a higher risk of *C. coli* during the summer (OR = 1.313) and in people older than 19 years (OR = 1.264). Living in a rural area was associated with a higher risk of infection (OR = 1.300). The 3rd study by Roux et al. (Roux et al. 2013) showed that female gender was a risk factor (OR = 1.940), which may be explained by females being more likely to prepare poultry in the home. The findings from these case-control/case-case studies (Bessell et al. 2012; Roux et al. 2013) indicate differences between the aetiology of *C. coli* and *C. jejuni* infections. The rurality of some of the cases (*C.coli* vs. controls and *C.coli* vs. *C. jejuni*) could indicate that PWS together with other environmental factors may contribute to the disease burden in rural areas.

The case-control and case-case studies presented in 6.3.6 and 6.3.7 suggest that environmental risk factors may

play an important role in the burden of *Campylobacter* disease. These studies demonstrated that genetic information obtained from the *Campylobacter* strains was important in source attribution and understanding the relative importance of risk factors. The importance of PWS to the disease burden was not determined in the majority of these models, except for the case-control study in N-E Scotland (Smith-Palmer & Cowden 2010). This study suggested that drinking water from PWS represents a risk for contacting campylobacteriosis, but the scale of the study was relatively small. Therefore, further epidemiological and typing studies of a similar type should be carried out.

4.7 Risk categorisation

The rationale for categorisation of PWS/hazard combination is presented in Appendix VI.

Disease incidence

The *Campylobacter* case-control study in N-E Scotland (Smith-Palmer & Cowden 2010) classifies PWS as one of the lowest impact (but not negligible) risk factors (8.2% in terms of disease prevention, when compared with the impact of eating outside the home (28.5%)). However, this was only a regional study, and the results cannot be extrapolated to the whole Scotland. There are also no other *Campylobacter* models which determine the incidence of disease by transmission pathway, source, or any other risk factor (e.g. food, drinking water from PWS, etc.).

The burden of disease caused by drinking water from private water supplies is unknown. Therefore, the overall incidence, from all sources, for each pathogen is used as an alternative way for risk categorisation (see Table A1 in Appendix VI). *Campylobacter* was categorised as the highest risk (rank 1) in terms of overall disease incidence for GI pathogens.

Several other disease ranking schemes would be possible if information on PWS contribution were available. A brief description of two of the extant rankings, which exclude PWS, is given below for information.

Attribution modelling performed in Scotland for human *Campylobacter* cases identifies chicken as the most important source of disease (41 to 46%) followed by ruminants (35 to 45%) and wild birds (16 to 19%) (Strachan et al. 2013). Pigs have a minor contribution (<0.2%). These results put chicken in the poll-position in terms of disease risk, followed by ruminants. There were no *Campylobacter* isolates sourced from PWS (or other environmental sources) to be included in this attribution model. Intensive sampling of PWS for *Campylobacter* would be necessary to accomplish this.

The Dutch case-case study (Mughini Gras et al. 2012) on human campylobacteriosis identified human strains which were of environmental origin by source attribution. Using only these strains, the study found that people suffering from a GI condition were at the highest risk (PAF 35%), compared with people swimming in a domestic pool (Mughini Gras et al. 2012)AF 28%). No information on consumption from PWS was available in this study. This model would be adaptable to Scotland, if case-control metadata (including information on consumption from PWS) were available.

Disease severity

The severity of disease from waterborne infections is also unknown. This is also required for risk categorisation but has not been done here. However, if it is assumed that the spectrum of disease obtained from all cases in Scotland is the same as those contracted from water then this can potentially be used in risk categorisation.

5.0 Scoping out microbiological risk assessments for *Campylobacter*

Table A.3 Scope out microbiological risk assessments for Campylobacter*

Steps	Variables†	Availability of data (Yes/No) and References	Comments
1. Pathogen sources	- prevalence of pathogens in sources	cattle (Yes), sheep (Yes), poultry (Yes), pigs (Yes), wild birds (Yes) All from (Ogden et al. 2009, and FSS iCamps reports	- surveillance at University of Aberdeen (2007-2015) - data available at farm level and individual animals for cattle, sheep and pigs, at abattoir for all animals (except wild birds) and from parks/ cities for wild birds
	- concentration of pathogen in faeces	cattle (Yes), sheep (Yes), poultry (Yes), pigs (Yes), wild birds (Yes) All from (Ogden et al. 2009, and FSS iCamps reports	- surveillance at University of Aberdeen (2007-2015) - data available at farm level and individual animals for cattle, sheep and pigs, at abattoir for all animals (except wild birds) and from parks/ cities for wild birds
2. Transport to PWS and Type of PWS	- transport variables	- See E. coli O157	- information available predominantly for commensal E. coli which can be potentially used as a surrogate for Campylobacter.
	- density of animals in the vicinity of PWS	cattle (Yes), sheep (Yes), pigs (Yes), poultry (Yes) wild birds (Yes) (Gibbons et al. 1993)	- domestic animals from EDINA Digimap at 2 x2 SqKm level agcensus.edina.ac.uk - density of wild birds need updated as is from 1991
	- position of PWS	Yes	-from DWQR and Local Authorities
	- type of PWS	Yes	- DWQR and Local Authority
	- prevalence in PWS	Yes (Smith-Palmer & Cowden 2010)	- survey in N-E Scotland
	- concentration in PWS	No	NA
3. Survival of pathogens in PWS (or other waters)	- temperature	Yes (unpublished)	- unpublished data at University of Aberdeen
4. Treatment	- proportion of treated PWS	Yes (MacRitchie et al. 2013)	- only for Grampian in 2009 -data needs updated - data needed at national level (Estimate: 80 % of type As; 35 % type B's - personal communication 2019 DWQR)
	- $\log_{10}$ reduction (treatment 1)	Yes (Hijnen et al. 2006)	The only treatment for which there is any reliable information is UV disinfection. Studies only tested to 4-log reduction.
	- prevalence before treatment	No	NA
	- prevalence after treatment	No	NA
	- concentration before treatment	No	NA
	- concentration after treatment	No	NA
5. Dose response (DR)	- dose ingested per glass of water	No	NA

Steps	Variables†	Availability of data (Yes/No) and References	Comments
	- dose response fitting parameters	Yes (Teunis et al. 2005)	Exponential parameter – (r)
	- probability of infection from drinking a glass of water	NA (will be generated on completion of the model) (Teunis et al. 2005)	Exponential model

***There is no microbial risk assessment model for *Campylobacter* infection from drinking water from PWS in Scotland. The main steps and variables needed to develop a model are given.

†The input variables are highlighted in blue text, the validation variables in purple, and the output variables in green. Missing data are marked with "No" red text.

Appendix IV. Indicator organisms

1.0 Hazard identification

1.1 The organism

Indicator organisms such as coliform bacteria, *E. coli*, heterotrophic microorganisms and enterococci are common indicators of faecal contamination of water (WHO 2011; Baker & Hegarty 1997). They can be used for a range of purposes including: surveillance of faecal pollution; determination of the efficacy of water filtration or disinfection and measuring the cleanliness of water distribution systems (WHO 2011). They in themselves are not hazards but if they are present, they give an indication that pathogenic micro-organisms may also be present.

Coliforms (excluding E. coli)

Coliform bacteria belong to the genera *Escherichia* (predominant genus), Enterobacter, Citrobacter and Klebsiella, comprising both aerobic and anaerobic, Gram-negative bacilli.

E. coli

Escherichia coli belong to the genera *Escherichia,* are Gram-negative, rod-shaped, facultatively anaerobic and thermotolerant bacteria, living in the intestines of humans and worm blooded animals (Singleton 2004). They can be differentiated from the other thermotolerant coliforms by the ability to produce indole from tryptophan and by the production of the enzyme β-glucuronidase (WHO 2011).

Heterotrophic microorganisms

Heterotrophic microorganisms include a broad range of bacteria and fungi that use organic (carbon-containing) compounds as a source of energy and carbon. They are used in heterotrophic plate counts (HPC), depending on their ability to multiply in a broad range of rich growth media, at specific temperatures and incubation times and without selective and inhibitory media.

Enterococci

The intestinal enterococci are part of the *Streptococcus* genus, being Gram-positive anaerobic bacteria and are relatively tolerant to HCl media and high pH levels.

1.2 Growth and survival

The growth and survival of indicator organisms is a broad subject (John & Rose 2005). Therefore, only a brief description is given for *Coliforms*, *E. coli*, *heterotrophic microorganisms* and *Enterococci* is provided.

Coliforms (excluding E. coli)

The coliforms are thermotolerant, grow in the presence of bile salts at high concentrations, ferment lactose at 44-45°C and produce aldehyde or acid in 24 hours at temperatures between 35-37°C (WHO 2011).

E. coli

E. coli grows in the same conditions as the coliforms, but also has the ability to produce indole from tryptophan and to produce β-glucuronidase.

Heterotrophic microorganisms

For the heterotrophic microorganisms there is a broad variety of growth media, the incubation temperatures can vary between 20 °C to 37 °C at incubation times ranging between few hours to one week.

Enterococci

Enterococci can be easily cultured using basic microbiological laboratory facilities, on selective media and incubation at 35–37 °C for 48 hours.

Further information on the growth and detection of indicator organisms has been reviewed by Ahmad (Ahmad et al. 2009). The factors affecting their survival in ground water have also been reviewed (John & Rose 2005).

1.3 Inactivation (Critical Control Points and Hurdles)

Coliforms (excluding E. coli)

Data (n=35) from ten studies have been used to compare the inactivation rates (log_{10}/day) of coliform bacteria in ground water at temperatures ranging between 3°C to 37 °C (John & Rose 2005). Variation of the inactivation rate was observed between studies (range = 0.01 to 1.5 log_{10}/day, geometric mean = 0.08. However, when only the data from the interquartile range (the middle 50%) was considered the inactivation rates ranged from 0.03 to 0.2 log_{10}/day (John & Rose 2005). This resulted in 90% inactivation times varying between 5 to 30 days.

E. coli

Inactivation rates (MPN/ml/hour, MPN – most probable number) were determined for *E. coli* in water microcosms, without and with presence of sand at various temperatures (4, 10, 14 and 25°C) (Sampson et al. 2006). The highest inactivation rate (8.33 x 10^5 MPN/ml/h) was obtained at 14 °C, in the absence of sand (the corresponding value in the presence of sand was lower-3.75 x 10^5 MPN/ml/h). The lowest inactivation rate (8.33 x 10^4 MPN/ml/h) was obtained at 4 °C in the absence of sand (the corresponding value in the presence of sand was 2.08 x 10^5 MPN/ml/h) (Sampson et al. 2006). The study by Sampson (Sampson et al. 2006) showed that both the temperature and the microcosms play an important role for the inactivation of *E. coli* in water.

Enterococci

Data (n=7) from seven studies were analysed to compare the inactivation rates of enterococci/faecal streptococci in ground water, for water temperatures ranging between 3°C to 22°C (John & Rose 2005). On average the inactivation of enterococci was similar with that of coliform bacteria, but the range was smaller (range = 0.01 to 0.8 log_{10}/day, geometric mean = 0.1, mean =0.3, stdev = 0.3, median = 0.2) (John, Rose 2005).

1.4 Sources

Coliforms (excluding E. coli)

Coliform bacteria are typically excreted in the faeces of humans and other warm blooded animals (WHO 2011). Many non *E. coli* coliforms can multiply in water and soil environments.

E. coli

E. coli is also excreted in the faeces of humans and other warm blooded animals, but can only survive in environmental waters (i.e. does not multiply, as water temperatures and nutrient conditions present in drinking-water distribution systems are unlikely to support the growth) and is rarely found in the absence of faecal contamination (WHO 2011).

Enterococci

The intestinal enterococci are also excreted in the faeces of humans and other warm-blooded animals. They are present in sewage and water environments polluted by animal faeces and untreated sewage, but they can be also found in soil in the absence of faecal contamination (WHO 2011).

Heterotrophic microorganisms

Heterotrophic microorganisms are usually part of the natural microbial flora (non-pathogenic) within water environments, being present in large numbers in raw waters (WHO 2011).

2.0 Hazard characteristics: adverse health effects

Indicator organisms do not generate adverse health effects.

3.0 Exposure Assessment

3.1 Contamination prevalence/ frequency, concentration, survival/ growth in water

In the NE Scotland study (Smith-Palmer & Cowden 2010) the prevalence of indicator organisms (coliforms, *E. coli* and enterococci) in public and private water supplies was determined (Table A.4). Although present in public waters, the prevalence of the indicator organisms was lower than in PWS. All, but one of the *Campylobacter* positive supplies tested positive for the three indicators organisms (coliforms, *E. coli* and enterococci). One *Campylobacter* positive supply was positive for coliforms only.

The concentration of these indicator organisms was also determined in the NE Scotland study (Smith-Palmer & Cowden 2010).

Table A.4 The prevalence of indicator organisms in public supplies and PWS (adapted from (Smith-Palmer, Cowden 2010)

Coliforms detected	Mains water from public supplies (%)	PWS (%)	Both (%)
Yes	16(1.7)	48(62)	1(25)
No	909(98)	29(38)	3(75)
Total	925	77	4
***E. coli* detected**			
Yes	0(0)	25(32)	1(25)
No	925(100)	52(68)	3(75)
Total	925	77	4
***Enterococci* detected**			
Yes	1(0.1)	25(32)	1(25)
No	922(99.9)	52(68)	3(75)
Total	925	77	4

Concentration of coliforms

In 75% (12/16) of the mains water from public supplies in which coliforms were detected the concentration was 1-9 cfu/ml. In seven (44%) of them only 1 cfu/ml was detected. The maximum coliform concentration (145 cfu/ml) was detected only in one mains water sample of public supply origin.

In 21% (10/48) of PWS samples in which coliforms were detected the concentration was 1-9 cfu/ml and in 29% (14/48) the concentration was >200 cfu/ml.

Concentration of E. coli

There was no *E. coli* detected in the mains water from public supplies.

In 52% (13/25) of PWS samples in which *E. coli* was detected the concentration was 1-9 cfu per ml and in 8% (2/25) the concentration was >200 cfu/ml. The raw data on *E. coli* in PWS available from Smith-Palmer et al. (Smith-Palmer, Cowden 2010) can be used as a proxy to determine the concentration of *E. coli* O157 in PWS (see subsection 5.4.2.2 in the main report and (Rotariu et al. 2012)).

Concentration of Enterococci

The single water sample from mains public supply which was positive for Enterococci had a concentration of 1 cfu/ml.

Among the 25 positive PWS samples the concentration of Enterococci ranged from 1 to 150 cfu/ml.

3.2 Dose ingested

Since indicator organisms do not cause disease there is not a need to estimate the dose ingested for the purposes of a dose response model.

Appendix V. *Cryptosporidium*

1.0 Hazard identification

1.1 The organism

Cryptosporidium spp. are coccidian protozoan parasites that infect a wide range of vertebrates, including humans stages, and produce thick-walled-hardy oocysts (WHO 2011; Arrowood 2002). The 4–6 µm oocysts are infectious when shed in faeces (WHO 2011; Arrowood 2002). To date >20 species of *Cryptosporidium* have been described, with *C. parvum* and *C. hominis* being the most common pathogens in humans worldwide (Xiao 2010). *C. hominis* is only found in humans, whilst *C. parvum* is a zoonotic pathogen found in both humans and animals (e.g. cattle and sheep).

1.2 Growth and survival

Cryptosporidium do not grow in the environment outside the host. Despite considerable effort to obtain *in vitro Cryptosporidium* cultivation, there are still obstacles in the maintenance of this protozoa in cultures for long periods of time (Arrowood 2002; Karanis & Aldeyarbi 2011; Zhang et al. 2012).

Outside the host, the temperature and relative humidity are known to have the most important effect on the survival of (oo)cysts (Alum et al. 2014). Although susceptible to higher (>15°C) environmental temperatures the *Cryptosporidium* oocysts can survive at temperatures of up to 64.2°C, although for short periods of time (<5 minutes) (Fayer 1994; King & Monis 2007). At low temperatures the pathogen survived at -22°C for up to 21 hours, although over 50% of the oocysts were not viable (King & Monis 2007: Robertson & Gjerde 2004). Desiccation appears to be one of the environmental stresses which is lethal for the survival of *Cryptosporidium* (e.g. after being air-dried for 2 h at room temperature only 3% of oocysts were viable) (King & Monis 2007; Robertson et al. 1992).

1.3 Inactivation (Critical Control Points and Hurdles)

Oocysts of *Cryptosporidium* are resistant to most chemical disinfectants and can survive for several months in cool and moist conditions (King & Monis 2007). However, oocyst infectivity can be destroyed by ammonia, formalin, freeze-drying, and exposure to temperatures < 0°C or >65°C (King & Monis 2007). Exposure to solar radiation reduces the viability of the oocysts (e.g. 90% reduction in viability after 3-days in marine water, 90% reduction in viability in tap water after one hour exposure to the highest UV index days) (Johnson et al. 1997; King et al. 2008). However, the majority of oocysts in bulk soil (including those in the top few centimetres) will be protected (Mawdsley et al. 1995; McGechan 2002).

1.4 Sources

Cryptosporidium spp. infect a wide range of vertebrate hosts including farmed animals (cattle, pigs, sheep, goats, horses), wild animals (deer, rabbits, rodents, foxes) and humans (Xiao 2010). For example, calves can excrete concentrations of up to 10^{10} oocysts per day (WHO 2011). *Cryptosporidium spp*. excreted from these reservoirs can be found throughout the environment including water (waste-, surface, drinking, recreational, non-recreational and ground water) (Agullo-Barcelo et al. 2012; Fuechslin et al. 2012; Gallas-Lindemann et al. 2013), soil (Boyer et al. 2009; Barwick et al. 2003; Santamaria et al. 2012), beach sand (Julio et al. 2012) and sewage (Bonadonna et al. 2002; Bukhari et al. 1997).

The prevalence of *Cryptosporidium* in different livestock (beef cattle – 80% (n=30), calves – 63.2% (n=57), sheep – 29.8% (n=47), lamb – 62.6% (n=158)) and wild red deer (80% (n=20)) was determined in a catchment area in the Cairngorms (Scotland), which supplied a public water supply that had a history of contamination (Wells et al., 2015). *C. parvum* prevalence as a percentage of total *Cryptosporidium* positive samples for each livestock type was: cattle - 96% (n=24), calves - 100% (n=36), sheep - 71% (n=14) and lambs – 89% (n=99). The predominant *Cryptosporidium* species in red deer was *C. parvum* (70% (n=13)). Prevalence and concentration of *Cryptosporidium* was also determined by immunofluorescence microscopy for a range of reservoirs (cattle, sheep, deer, bird, foxes, mice, rats) and environments (dirty water, manure, spread slurry) present on dairy farms in England and Wales (Smith et al. 2014). It is important to note that the prevalence of *Cryptosporidium* in dairy cattle obtained in England and Wales was much lower (10.2% (95%CI 9.4 – 11.1)) (Smith et al. 2014) than the prevalence (80%) found in the beef cattle from the Scottish study (Wells, Shaw et al. 2015). The prevalence of *Cryptosporidium* in sheep from England and Wales was also smaller (4.1 (95%CI 0.6 – 7.7)) (Smith et al. 2014) than the prevalence in sheep found in the Scottish study (29.8%) (Wells et al. 2015). Seventy-five percent (75%) of the positive dairy cattle and 80% of the positive sheep in the English study had low concentrations (10^3 to 10^4 oocysts/g) of *Cryptosporidium* in their faeces, whilst only 8.1% of positive cattle had high concentrations (>2 x 10^6 oocysts/g) and none of the positive sheep (Smith et al. 2014).

2.0 Hazard characterisation: adverse health effects

2.1 Disease symptoms

Cryptosporidium spp. are recognized as waterborne parasites of human health significance that have a worldwide distribution (Karanis et al. 2007). One of the most frequent symptoms of cryptosporidiosis is watery diarrhoea (sometimes profuse and prolonged) (Current & Garcia 1991; Meinhardt et al. 1996). Together with diarrhoea, other symptoms such as nausea, vomiting, abdominal cramps, low-grade fever and pain were observed in patients and healthy volunteers (DuPont et al. 1995; Bouzid et al. 2013). Infrequent symptoms include: malaise; headache; myalgia; weakness and anorexia (Current & Garcia 1991). The severity of cryptosporidiosis can vary from asymptomatic shedding or self-limiting (up to 2 to 3 weeks) to being life-threatening in immunocompromised people (Bouzid et al. 2013).

2.2 Dose response

Several dose response models have been developed for cryptosporidiosis (Haas et al. 2000; Gale 2001; Pujol et al. 2009). These studies used dose-response data from feeding studies in human volunteers (DuPont et al. 1995). Hass and colleagues (Haas et al. 2000), used the exponential model and obtained a best fit parameter of r = 0.0042, resulting in an ID_{50} = 165 oocysts (95%CI – 85 to 200 oocysts). Teunis and colleagues (Teunis et al. 2002a; Teunis et al. 2002b) developed two other dose-response models, an extended exponential model, using four fitting parameters, which took into account the variation between hosts, and a hypergeometric model to account for the variation between isolates.

2.3 Susceptible population

Immunocompromised people (e.g. HIV/AIDS patients with T cell counts) are regarded as being at the greatest risk of cryptosporidiosis (Casadevall & Pirofski 2018; Crawford & Vermund 1988; O'Donoghue1995; Soave et al. 1984). AIDS patients with CD4 cells counts <50 had the most severe disease, whilst patients with larger CD4 counts had self-limited cryptosporidiosis (Bouzid et al. 2013; Flanigan et al. 1992). Children with malaria were also found being at higher risk in Nigeria (Molloy et al. 2011) as well as children in poverty at the Mexico-Texas border (Leach et al. 2000). Also, mothers and infants 0 to 6 years old were at greater risk in rural communities from undeveloped countries (Pedersen et al., 2014) as well as female children (Laubach et al. 2004). However, the risk coming from public water supply in Brazil was higher in adults than in children.

In Scotland the incidence of both *C. hominis* (23.2 cases/100,000 people) and *C. parvum* (27.0 cases/100,000 people) was higher in children <4 years old, than in other age groups, suggesting a likely greater susceptibility and/or a higher exposure in this age group (Pollock et al., 2010). Also, during 2012-2013 the number of *C. parvum* cases was higher (n = 153) in the health boards having a higher rural population, including Grampian, Fife and Ayrshire/Arran, compared with the number of cases (n = 41) in Glasgow and Lothian, which are the two largest urban settings in Scotland (Deshpande et al. 2015; Deshpande et al. 2015). This was also found in a previous study, which highlighted that *C. parvum* was more prevalent in areas with lower population density, in those with a higher density of ruminant livestock, reflecting the zoonotic nature of the infection, and in areas with higher density of PWS, reflecting possible exposure through drinking water from these sources (Pollock et al. 2010).

2.4 Particular subtypes found in both humans and PWS

Cryptosporidium from Scottish waters (n=1,042 - 456 from raw waters and 586 from drinking waters (type not specified)), were analysed to detect either human-infectious or non-human-infectious *Cryptosporidium* oocysts (Nichols et al. 2010). The most common human-infectious *Cryptosporidium* species occurring in drinking water samples were *C. parvum* (4.3%) and *C. hominis* (1.3%). Other species detected in drinking waters were *C. ubiquitum* (12.6%) and *C. muris* (0.3%), but these occur rarely in humans. Real time PCR speciation of *Cryptosporidium* oocysts obtained from human cases in Scotland during 2012-2013, found that 42% (287/445 speciated) of cases were *C. hominis* and 57.5% were *C. parvum,* respectively (Deshpande et al. 2015; Deshpande et al. 2015). Similar proportions (49% *C. hominis* and 51%) were observed in an earlier Scottish study (Pollock et al. 2010). There is no data in these studies about the source of contamination. However, the study by Pollock and colleagues (Pollock et al. 2010) found a positive association between *C. parvum* occurrence and the density of PWS (i.e. number of PWS/100 people).

3.0 Exposure assessment

3.1 Contamination prevalence/ frequency, concentration, survival/ growth in water

One study across the UK reports *Cryptosporidium* prevalence and concentration data from intensive monitoring of commercial PWS (Kay et al. 2007). Daily sampling of potable water from seven PWS (two in each of England, Scotland and Wales and one in Northern Ireland, comprising six treated and one untreated) was performed using large volume filtration. This study was performed over two six weeks periods in 2000 during spring (May and June) and autumn (end of September to mid-November). During the whole study period *Cryptosporidium* was detected in all seven PWS, the minimum prevalence being 2.4% (1/41) and maximum 91% (37/41). Two PWS were negative at springtime, but positive in the autumn. The untreated PWS, which had a spring-fed surface water source, was positive for *Cryptosporidium* during both sampling periods (33.3% in spring and 56% in autumn, respectively). This PWS also had high prevalence of faecal indicator organisms.

The maximum *Cryptosporidium* concentration at each PWS and sampling period was reported. Of the PWS that were found to be positive at least once, the maximum concentration varied between 1 oocyst/1000 litres of water and 2848 oocysts/litre. In terms of QMRA the distributions of concentration would be desirable, although *Uniform (0, max)* distributions could be an alternative to make use of the maximum values. Another drawback of the data supplied in the study (Kay, Watkins et al. 2007) is the lack of speciation of the *Cryptosporidium* (Pachepsky et al. 2006; Walker & Stedinger 1999).

Inactivation of *Cryptosporidium* in the environment (e.g. water, soil and faeces) follows an exponential decrease with time (Pachepsky et al. 2006; Walker & Stedinger 1999). This can be fitted by a mathematical model which is described below for completeness.

$$N(t) = N_0 e^{-K_T t} \qquad (7)$$

where N_0 and $N(t)$ represent the oocyst numbers at time zero and time t. The die-off coefficient K_T changes with various environmental factors, with temperature being regarded as one of the most important (Peng, Murphy et al. 2008). An exponential relationship has been proposed for the change of K_T with the temperature, T

$$K_T = K_4 e^{\lambda (T-4)} \qquad (8)$$

where K_4 is the die-off coefficient at 4°C and λ a fitting coefficient measured in ($^{\circ}C^{-1}$). These coefficients were calculated for sterile water, river water, sea water and raw surface water, but no data exist for PWS (Peng et al. 2008).

3.2 Dose ingested

Cryptosporidium data in PWS from the UK (Kay et al. 2007) was correlated with corresponding *E. coli* data using log-log regression (Hunter et al. 2011). Combining the daily consumption of unboiled tap water in England, it was possible to calculate the daily ingested dose of *Cryptosporidium* from PWS (mean 0.0053, median 0.0012, lower 90% CI 0.00014, upper 90% CI 0.012 (Hunter et al. 2011)). There have been no estimates of the *Cryptosporidium* daily ingested dose from PWS in Scotland.

4.0 Risk characterisation

4.1 Incidence – Scotland

Figure 3 (see section B3 of main report) presents the incidence of cryptosporidiosis in Scotland during 2009 – 2013. There is no temporal trend (linear regression analysis, P - 0.08) and the average incidence for the last 14 years is 11.5 cases/100,000 people (95%CI – 10.2 to 12.8).

4.2 Clinical consequences of infection

Data on Scottish hospitalisation rates of cryptosporidiosis have only been published in part (Pollock & Hawkins 2015). In the early 90's the average hospitalisation rate was 1.3 hospital admissions per 100,000 population (Robertson 1996). Of those hospitalised 28% were children < 5 years of age which spent in hospital between 1 to 20 days (median – 4 days) (Robertson 1996). During 2011-2014 the average hospitalisation rate for *Cryptosporidium* in Scotland was similar, being 1.3 hospital admissions per 100,000 population (Pollock & Hawkins 2015). The same study reported that 12% of cases are hospitalised and 17% of patients have subsequent hospital admissions (average – 2 admissions/ patient) (Pollock & Hawkins 2015).

During the late 80's a survey in England and Wales reported that 19% (241/1283) of cryptosporidiosis cases who completed a questionnaire were admitted to hospital. This comprised 26 (11%) who were infants, 96 (40%) 1-4 years old, 49 (20%) 5-14 years old and 11 (4.6%) aged 65 and over (Palmer 1990). Severe illness (watery diarrhoea with at least six (median) bowel motions per 24 hours, abdominal cramps, fever, and vomiting) was reported in 8% (107/1283) of all patients, with 3.5% (45/1283) having prolonged diarrhoea (>21 days).

In developing countries cryptosporidiosis the parasite was reported in 5 - 10% of diarrheal stool samples from

children (Checkley et al. 2015; Khan et al. 2004). It is also associated with long duration of diarrhoea (5 - 15 days) in children, when compared with other GI pathogens (1 - 4 days) (Bern et al. 2002; Khan et al. 2004). In Brazil a case-control study in children <1 year of age found that cryptosporidiosis cases were associated with higher diarrheal morbidity when compared with non-*Cryptosporidium* infected controls (OR – 3.4, P<0.001) (Agnew et al. 1998; Checkley et al. 2015). Also, in India 1 in 6 to 11 children (< 2 years old) have an episode of diarrhoea caused by *Cryptosporidium* with 1 in 169 to 633 being hospitalised, and 1 in 2890 to 7247 dying (Sarkar et al. 2014).

In immunocompromised people (e.g. HIV-infected, elderly, patients undergoing chemotherapy or suffering transplants) the disease can be severe, i.e. the patients may develop chronic diarrhoea and atypical gastrointestinal disease (e.g. cholangitis, cholecystitis, hepatitis) (Chalmers 2008; Kortbeek 2009).

4.3 Outbreaks

In the 10 year period 2005 to 2014, there were 20 *Cryptosporidium* outbreaks in Scotland which were reported to Health Protection Scotland (Anonymous 2016). These outbreaks accounted for approximately 305 cases (~15 cases/outbreak) which is approximately 5% of the total number (5800) of *Cryptosporidium* cases. During this time in Scotland there were five *Cryptosporidium* outbreaks related to water which were reported to HPS (Anonymous 2016). Four of these outbreaks had swimming pools as probable sources and for one outbreak there was no further information on the water source (Smith-Palmer & Cowden 2013). There is no published information of any *Cryptosporidium* outbreaks related to PWS in Scotland in the last 10 years.

Outbreaks related to drinking water, which were common in the 1990s, have declined since 2000 and are now uncommon in the UK, because of the improvements in mains water treatment (Anonymous, 2016). In England a mixed *Campylobacter/Cryptosporidium* outbreak was associated with an untreated PWS in the early 90's (Duke et al. 1996) (see 6.4.4).

4.4 Sporadic cases

In Scotland the majority of *Cryptosporidium* cases are sporadic, with a total of 5802 cases reported to HPS between 2005 to 2014 of which only 305 are associated with an outbreak (Anonymous 2016). An epidemiological study carried out on 560 sporadic *Cryptosporidium* cases reported to HPS between June 2005 and July 2007 found a positive association between the occurrence of *C. parvum* (n=284) and the density of PWS (i.e. number of PWS per 100 people) (P<0.001) (Pollock et al. 2010). There are no other studies in Scotland which relate the occurrence of sporadic cryptosporidiosis to PWS.

4.5 Risk assessments

Generalised spatial linear Poisson model in Scotland

The linear Poisson model was based on spatial *C. parvum* epidemiological data collected in Scotland between 2005-07 (see subsection 6.2 in the main report) (Pollock et al. 2010). A variety of risk factors (including the density of PWS, geographical location, farms to people ration, human density, sheep density) were considered and the relative risks and corresponding 95% confidence intervals were determined.

BQMRA of PWS in England

A Bayesian quantitative microbial risk assessment (BQMRA) was developed in England to estimate the probability of infection (daily and per year) with *Cryptosporidium* from drinking water from PWS (Hunter et al. 2011). Exposure was modelled using the daily consumption of unboiled tap water as a Poisson distribution with a mean of 533 ml/day. The concentration of the pathogen was determined by log-log linear regression analysis using *Cryptosporidium* and *E.coli* data from seven PWS in the UK (Kay et al., 2007) and *E.coli* concentration in drinking tap water collected from 11,233 PWS in England between 1995 to 2003 (Richardson et al. 2009). The daily probability of infection per person was calculated using the Beta-Poisson model ($\alpha = 0.115$, $\beta = 0.176$) (see eq4. in 4.1.2) (Teunis et al. 2002a; Teunis et al. 2002b). The probability of one or more infections per person per year was modelled as a Beta-Binomial function,

$$P_{inf/year} = 1 - (1 - P_{inf/day})^{365} \qquad (14)$$

A sensitivity analysis was performed to determine the impact of the variation in the input variables.

4.6 Qualitative/Quantitative estimate of risk

Generalised spatial linear Poisson model in Scotland

This model (Pollock et al. 2010) found a positive association between cases of *C. parvum* and the density of PWS (P<0.001). The relative risk (RR-1.060, 95%CI 1.032-1.088) showed that there were more cases in areas with larger number of PWS per person. Three other risk factors (geographical location, farms to people ratio and sheep density) were positively associated with the disease, whilst population density was protective.

BQMRA of PWS in England

The average daily probability of infection from an English

PWS was estimated to be 0.0023 (median = 0.00079, 90%CI = (0.000094, 0.0079)) (Hunter et al. 2011). The average annual probability of one or more infection per person was estimated to be 0.57 (median = 0.25, 90%CI= (0.034, 0.94)) (Hunter et al. 2011). The arithmetic average daily risk was an order of magnitude higher than the median, suggesting that there were some high risk PWS. These PWS could be detected by comparing the daily probability of infection with the upper 90% confidence limit of the distribution for this probability.

4.7 Risk categorisation

The rationale for categorisation of PWS/hazard combinations is presented in Appendix VI.

Disease incidence

The burden of disease caused by drinking water from private water supplies is unknown. There are also no *Cryptosporidium* models which determine the incidence of disease by transmission pathway, source, or any other risk factor (e.g. food, drinking water from PWS, etc.). For example, the generalised linear Poisson model, developed by Pollock and colleagues (Pollock et al. 2010), considered PWS as being a risk factor for *Cryptosporidium*, along with three other factors (geographical location, farms to people ratio and sheep density). However, there is no data supplied in this model to determine and rank the incidence of the disease by each risk factor. Therefore, the overall incidence, from all sources, for each pathogen is used for risk categorisation (see Table A1 in Appendix VI). *Cryptosporidium* was categorised as one of the lowest risks (rank 4) in terms of overall disease incidence.

Disease severity

The severity of disease from waterborne infections is also unknown. This is also required for risk categorisation but has not been done here. However, if it is assumed that the spectrum of disease obtained from all cases in Scotland is the same as those contracted from water then this can potentially be used in risk categorisation.

5.0 Scoping out microbiological risk assessments for *Cryptosporidium*

Table A.5 Scope out microbiological risk assessments for Cryptosporidium****

Steps	Variables†	Availability of data (Yes/No) and References	Comments
1. Pathogen sources	- prevalence of pathogens in sources	cattle (Yes), (Sturdee et al. 2003; Brook et al. 2008)	- includes dairy cattle and calves and a small number of horses and wild animals
		sheep (Yes), (Smith et al. 2014; Sturdee et al. 2003; Connelly et al. 2013)	
		horses (Yes), (Sturdee et al. 2003)	
		wild animals (Yes) (Smith et al. 2014)	
	- concentration of pathogen in faeces	cattle (Yes), (Sturdee et al. 2003; Brook et al. 2008)	- includes dairy cattle and calves and a small number of horses and wild animals
		sheep (Yes), (Smith et al. 2014; Sturdee et al. 2003; Connelly et al. 2013)	
		horses (Yes), (Sturdee et al. 2003)	
		wild animals (Yes) (Smith et al. 2014)	
2. Transport to PWS and Type of PWS	- transport variables	- See *E. coli* O157	- information available predominantly for commensal E. coli. A literature research would need to be carried out for cryptosporidium oocysts or particle transport with similar physical properties.
	- density of animals in the vicinity of PWS	cattle (Yes), sheep (Yes), horses (Yes) all from Ref, wild animals (No)	- domestic animals from EDINA Digimap at 2 x2 SqKm level agcensus.edina.ac.uk
	- position of PWS	Yes	-from DWQR and Local Authorities
	- type of PWS	Yes	- DWQR and Local Authority
	- prevalence in PWS	Yes (Hunter et al. 2011)	- data from UK survey

	- concentration in PWS	Yes (Hunter et al. 2011)	- data for 7 PWS in UK including 2 PWS from Scotland
3. Survival of pathogens in PWS (or other waters)	- temperature	Yes (Walker, Stedinger 1999; Pachepsky et al. 2006)	- in water, not particularly PWS
4. Treatment	- proportion of treated PWS	Yes (MacRitchie et al. 2013)	- only for Grampian in 2009 -data needs updated - data needed at national level (Estimate: 80 % of type As; 35 % type B's - personal communication 2019 DWQR)
	- log_{10} reduction (treatment 1)	Partially (Bukhari et al. 1999; Zimmer & Slawson 2002)	The only treatment for which there are reliable data is UV disinfection. One study used a dose of 50 mJ/cm^2 which is greater than the usual dose applied to PWS. The other study only considered buffered saline, not real water. The actual log reduction for well-functioning UV on a private supply is likely to be somewhere in between the values given by these studies.
	- log_{10} reduction (treatment 2)	(Abbaszadegan et al. 1997)	>36-log reduction protozoan cysts with a point of use activated carbon plus UV filter (UV fluence not given).
	- prevalence before treatment	No	NA
	- prevalence after treatment	No	NA
	- concentration before treatment	No	NA
	- concentration after treatment	No	NA
5. Dose response (DR)	- dose ingested per glass of water	No	NA
	- dose response fitting parameters	Yes (Haas 2000)	(α, β), Beta –Poisson parameters
	- probability of infection from drinking a glass of water	NA (will be generated on completion of the model) (Haas 2000)	Beta-Poisson model

****There is no microbial risk assessment model for *Cryptosporidium* infection from drinking water from PWS in Scotland. The main steps and variables needed to develop a model are given.

†The input variables are highlighted in blue text, the validation variables in purple, and the output variables in green. Missing data are marked with "No" red text.

APPENDIX VI: Methods to categorise/rank PWS risk by pathogen

The assignment of a risk category for a private water/ hazard combination would use two criteria: the incidence and the severity of disease.

1. Incidence

The incidence would be an estimate of the waterborne (PWS) disease rate (cases/100,000 population) due to an individual hazard (e.g. *Cryptosporidium*). The incidence would need to be estimated for each waterborne pathogen and the incidence data ranked/categorised. Unfortunately, there is no waterborne disease incidence data for Scotland to do this categorisation. Therefore, to illustrate by means of example overall incidence rates from Scotland are used (Anonymous 2016).

Table A.6 shows the range of incidence rates in Scotland during the ten years (2005-2014) for the pathogens being considered in this study. The incidence data was ranked into 5 categories with *Campylobacter* having the highest incidence being ranked 1.

Table A.6 The range of incidence rates in Scotland in the ten years (2005-2014) for the pathogens of concern considered in this study.

Organism	Incidence range (cases/100,000)	Rank
Salmonella	13.4 - 22.5	3
Shigella	0.9 - 3.3	5
VTEC	3.1 - 5.1	5
Campylobacter	85.9 - 126	1
Norovirus	24.4 - 59.5	2
Rotavirus	6.5 - 35	3
Adenovirus	12.6 - 33.3	3
Cryptosporidium	8.1 - 13.9	4
Giardia	3.1 - 4.3	5

The incidence associated with PWS could also be estimated using the following methods:

i. pathway of disease transmission using QMRAs or regression analysis techniques (Rotariu et al. 2012);

ii. source attribution (at the level of the PWS), using genetic/phenotypic information (e.g. MLST typing) to type the pathogens and Bayesian inference to calculate the proportion for a pathogen to occur from a specific source (Pires et al. 2009; Strachan et al. 2013). This can also be combined with regression analysis (Mughini Gras et al. 2012; Roux et al. 2013).

iii. risk factor approach (e.g. food consumption, travel, PWS, etc.), using case-control studies, from which the proportion of cases in population and the relative risk between cohorts (i.e. cases and controls) can be determined. From these models the population attributable fractions can be calculated, which will allow an estimate of the disease that is associated with for example private water supplies (Rockhill et al. 1998).

2. Severity

Severity is related to the range (and frequency) of sequelae associated with the hazard. The outcomes of infection can be defined as (Lake et al. 2000):

- death

- hospitalised and long-term illness (e.g. HUS, reactive arthritis, GBS)

- hospitalised and recover

- visit a GP but not hospitalised

- does not visit a GP

The first three outcomes can be considered as severe outcomes (Lake et al. 2000).

Severity data can be used across all cases of each disease (e.g. campylobacteriosis) in the human population where it can be assumed that this disease spectrum is representative to that from PWS. Or specific disease severity data from cases of disease from PWS can be used where that becomes available.

A ranking score can be generated for disease (similar to incidence described above). The multiplication of the incidence and severity scores can then be used as an indicator of overall risk.

An alternative approach is to employ an economic approach which involves calculating DALYs and other costs of disease to turn this into monetary values. This is described in some more detail in section 7.3 in the main report 'Adverse Economic Effects from Infection with pathogens from PWS'.

APPENDIX VII: Risk characterisation for *Salmonella*, *E. coli* O157, *Campylobacter* and *Cryptosporidium* infection from PWS

Table A.7 Risk characterisation‡ for Salmonella, E. coli O157, Campylobacter and Cryptosporidium infection from PWS.

Steps	Variables†	Availability of data (Yes/No) and References	Comments
6. Consumption	- number of glasses per person/year	Yes (MacRitchie et al. 2013)	- survey at University of Aberdeen – only data for Grampian but may be extrapolated for rest of Scotland
	- amount of water in a glass	Yes (Hunter et al. 2011)	- based on an English survey
	- number of glasses / years by specific age groups	Yes (MacRitchie et al. 2013)	- survey at University of Aberdeen – only data for Grampian but may be extrapolated for rest of Scotland
	- number of people on PWS	Yes (Rotariu et al. 2012) and available from local authorities	- Estimated (based on no of properties on PWS)
7. Disease burden in Scotland *Salmonella*	- Number of cases per year	NA (will be generated on completion of the model)	Not done
E. coli O157	- Number of cases per year	NA (generated (Rotariu et al. 2012) but needs updated to include the transport step and treatment of PWS)	Done, but needs updated
Campylobacter	- Number of cases per year	NA (will be generated on completion of the model)	Not done
Cryptosporidium	- Number of cases per year (output variable)	NA (will be generated on completion of the model)	Not done

†The input variables are highlighted in blue text, the validation variables in purple, and the output variables in green. Missing data are marked with "No" red text.

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