University of East Anglia,

WRc (Water Research Centre)

and

Drinking Water Inspectorate

RISK ASSESSMENT OF VTEC INFECTIONS IN ENGLISH AND WELSH DRINKING WATER

report for contract DWI/ 70/2/256

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Summary

Shiga toxin positive Toxin positive *E. coli* O157 and related STEC strains are amongst the most serious of waterborne pathogens that pose a threat to drinking water supplies. The concern is particularly due to that fact that about 10% of cases in children go onto develop haemolytic uraemic syndrome and also the high mortality rates in the very young and very old. Whilst most concern relates to *E. coli* O157 other STEC strains are increasingly being recognised, but as yet they are less commonly identified as being associated with waterborne outbreaks. The recent emergence of *E. coli* O104:H4 in Germany raised especially great concerns due to the high fatality rate in previously health adults. Although *E. coli* O157 has been reported to cause outbreaks associated with drinking water in the UK and elsewhere, there is still little information about how common sporadic waterborne infections may be. In the few well conducted case control studies of sporadic disease, potable water from public supplies has not been implicated, although unchlorinated surface water has been identified as a risk factor. This paper reports a study aimed at trying to estimate the risk of STEC infections due to drinking water in the absence of detectable outbreaks of disease. The report follows a series of studies that included systematic reviews, surveys of water utilities and private supplies and a quantitative microbiological risk assessment with the ultimate aim of determining the risk to health associated with this pathogen in English and Welsh drinking water supplies

We report a systematic review of the literature to determine the prevalence of *E. coli* O157 in raw waters and in livestock that that may be sources of contamination of such raw water. There was a dearth of studies that reported on concentration of *E. coli* O157 in raw waters or indeed in water intended for consumption. We were however, able to identify several papers that addressed the detection of *E. coli* O157 in livestock, though most gave only presence-absence data. We were able to find one PhD thesis that derived a distribution of counts in positive livestock. In addition we reviewed evidence on the susceptibility of *E. coli* O157 to disinfection and concluded that the evidence suggests that *E. coli* O157 has the same susceptibility as indicator *E. coli.*

Several water utilities were contacted about their disinfection policies and more detailed information obtained on a number of Water Treatment Works. Chlorination policies differed from utility to utility but ranged from A Ct of 15 to 60 mg.min L^{-1} depending on water quality. Across England and Wales DWI recorded on average one chlorination failure per month, the majority of which lasted less than 24 hours (median 6 hours). During 2010 there were 38 reported breaches in the integrity of water mains.

The results of a sanitary survey of 270 private water supplies in Herefordshire and East Anglia are also reported. Only 40% of owners reported using any disinfection and of these only 59% kept a record of water treatment maintenance. There were in addition a range of other problems such as

on site sewerage, proximity of livestock and unsatisfactory repair of the systems that would pose an increased risk of contamination.

A quantitative microbial risk assessment (QMRA) was conducted, using data collected from the literature, water utilities and the drinking water inspectorate. We conducted a separate QMRA for private supplies and for 13 randomly selected public water supplies owned by four different water companies. The risk assessment was based on data of indicator *E. coli* concentrations in tap water for private supplies (obtained from DWI) and in raw water for the public supplies (obtained from the utilities). The O157: indicator *E. coli* ratio was estimated for each catchment from the known number of livestock occupying the catchment, the estimate of the proportion excreting and a model of shedding intensity. Daily water consumption was modelled from the recent DWI water consumption survey and the risk model was the Beta Poisson model with parameters according to Teunis et al. (2004). Risk was calculated by MonteCarlo modelling using @Risk5.

The mean annual risk in adults consuming unboiled tap water from private supplies is 5 cases per 10000 person years. However, almost all of this risk is experienced by people whose water quality fails the statutory *E. coli* standard. When the modelling was restricted to those supplies that complied with current standards the mean annual risk was estimated to be only 0.8 cases per 10000 person years. The annual risk in the 13 water utility sites range from 0.00065 cases per 10000 person years in adults to 0.69 cases per 10000 person years. All water utilities are able to provide water with an annual risk of less than 1 per 10000 person years. In the model that included one day chlorination failure risk remained than 1 per 10000 person years. It is likely that the estimated risks for the public water supplies are over-estimates as we used a very conservative estimate of chlorination.

1 Introduction

Of all the newly emerged potentially waterborne diarrhoeal pathogens of the past few decades *E. coli* O157 is probably the most important. The importance of this pathogen arises from the severity of the disease especially in the young and the elderly. The virulence of this organism comes from the combination of the intimate attachment of the organism to the gut epithelium and the subsequent the dissolution of the microvilli with the production of Shiga-like toxins (Hunter 2003). A particular issue is the subsequent development of haemolytic uraemic syndrome (HUS) in about 10% of children. The pathogen also has a low infectious dose. This class of pathogen has been given several different names: in the UK it has frequently been called Verocytotoxigenic *E. coli* (VTEC) whilst in the US it is usually called Enterohaemorrhagic *E. coli* (EHEC), more recently the term Shiga toxin producing *E. coli* (STEC) is gaining ground.

STEC are found in the intestines of several animal species, especially cattle. Infection of humans can follow direct faecal-oral spread from infected animals or other humans, or be related to contamination of food or water. Outbreaks have been described due to person-to-person transmission, zoonotic, food and water borne infections (Hunter 2003).

This study establishes risks to consumers of UK water supplies from *E. coli* O157 and other STEC in drinking water. Separate analyses have been conducted for people consuming mains water and for people reliant on private water supplies. The objectives are:

1) to review data from the grey and published literature on the prevalence of *E coli* O157 in raw water sources.

2) in the absence of robust data on prevalence in raw water, make an assessment of likely levels based on all possible input into catchments

3) to review data from the grey and published literature on the susceptibility of *E. coli* O157 to disinfection regimes, including the relative susceptibility of *E coli* O157 and existing indicators

4) gather data on current disinfection regimes used in public and private water supplies from a representative selection of water companies and local authorities in England and Wales

5) gather data on the level of possible faecal contamination in sources used in public and private water supplies from a representative selection of water companies and local authorities in England and Wales

6) use the data gathered in objectives 1) - 5) and knowledge of infectivity to quantify any risks arising in terms of risks to public health from waterborne *E coli* O157 arising from normally operated public and private supplies.

7) gather data from a representative selection of water companies to assess frequency of impairment of disinfection and ingress in distribution

8) use the data gathered in objectives 5 to quantify any risks arising in terms of risks to public health from waterborne *E coli* O157 arising from impairment of disinfection and ingress in distribution.

9) prepare a report of the findings that appraises the Inspectorate's position, quantifies the risk and advises on possible future research or monitoring.

2 Presence of *E. coli* O157 in livestock and in raw waters a Systematic Review

In order to inform the model, a systematic review of the literature was conducted to identify the abundance and prevalence of *E. coli* O157 and other STEC in raw water sources. Early in the review it was noted that robust information on the presence of *E. coli* O157 and other STEC in raw water sources was relatively scarce. Therefore, the review was extended to identify the likely levels based upon all inputs into catchments. This was based predominantly on levels in manure voided to land by livestock. In terms of livestock levels the search strategy was restricted to the faecal material of sheep, swine and cattle. Other livestock such as horses were excluded from the search due their low shedding rates and low density in England and Wales.

2.1 Methods of the review

2.1.1 Search strategy

A search strategy was designed to identify relevant papers. The strategy used both free text (searching in title, abstract and keywords) and database specific INDEX terms. To improve the specificity of the search, terms relating to Livestock (A) were combined with Manure (B) using the Boolean operator AND. The terms Livestock and Manure and Water (C) were combined with the Boolean operator OR, and these terms were subsequently combined with *E. coli O157* (D) using AND.

A. Livestock (including: ovine, bovine etc)
B. Manure (manure, faeces etc)
C. Water (water, groundwater, river, lake etc)
D. *E. coli O157* (VTEC, EHEC, *E. coli* O157 etc)

((A AND B) OR C) AND D

A full list of terms used in the search strategy can be found in appendix 1. The search strategy was applied to the following databases: Medline, Embase, Scopus, ISI Web of Knowledge, Geobase, Biosis, ProQuest and VHL (for LILACS, REP, WHO, PAHO etc).

2.1.2 Water criteria

The following exclusion criteria were applied to studies reporting *E. coli* O157 in water: water quality in non-industrialised countries; water quality prior to and/or following an outbreak of infectious intestinal disease.

2.1.3 Livestock criteria

The following inclusion criteria were applied to articles relating to *E. coli O157* in livestock: published after the year 2000 (in order to obtain an up to date estimate of prevalence); cross-sectional study conducted in England, Wales and Great Britain; principal aim to determine prevalence (excluding experimental intervention studies and studies developing diagnostic tests).

The following exclusion criteria were applied: the farm and/or animal was selected on the basis of being O157 positive; prevalence was estimated during/following an outbreak of infectious intestinal disease in humans; microbial analysis was based on samples derived from 'waste' rather than faecal material (for example, samples including bedding material and urine).

2.1.4 Screening of titles and abstracts

Database search results were exported in separate files and imported into a combined Endnote library (totalling 16670 records). Duplicates were subsequently removed leaving 5879 titles and abstracts to screen. One reviewer screened the titles and abstracts to remove articles completely out of scope (aiming to be very inclusive), leaving 2252 titles and abstracts to be screened independently by two reviewers. The two independent reviewers met and any discrepancies for study inclusion were resolved. Of the 124 full text articles obtained, 36 publications met the inclusion criteria of which there were 33 independent studies. Thirty-one publications (29 unique studies) related to water and 5 (4 unique studies) to livestock. Figure 1 is a flow diagram of the screening and selection process for articles included in this review. Appendix 2 lists the publications which were reviewed in full text and the outcome of selection including reasons for exclusion. Data extraction was completed for all water and livestock publications meeting the inclusion criteria.

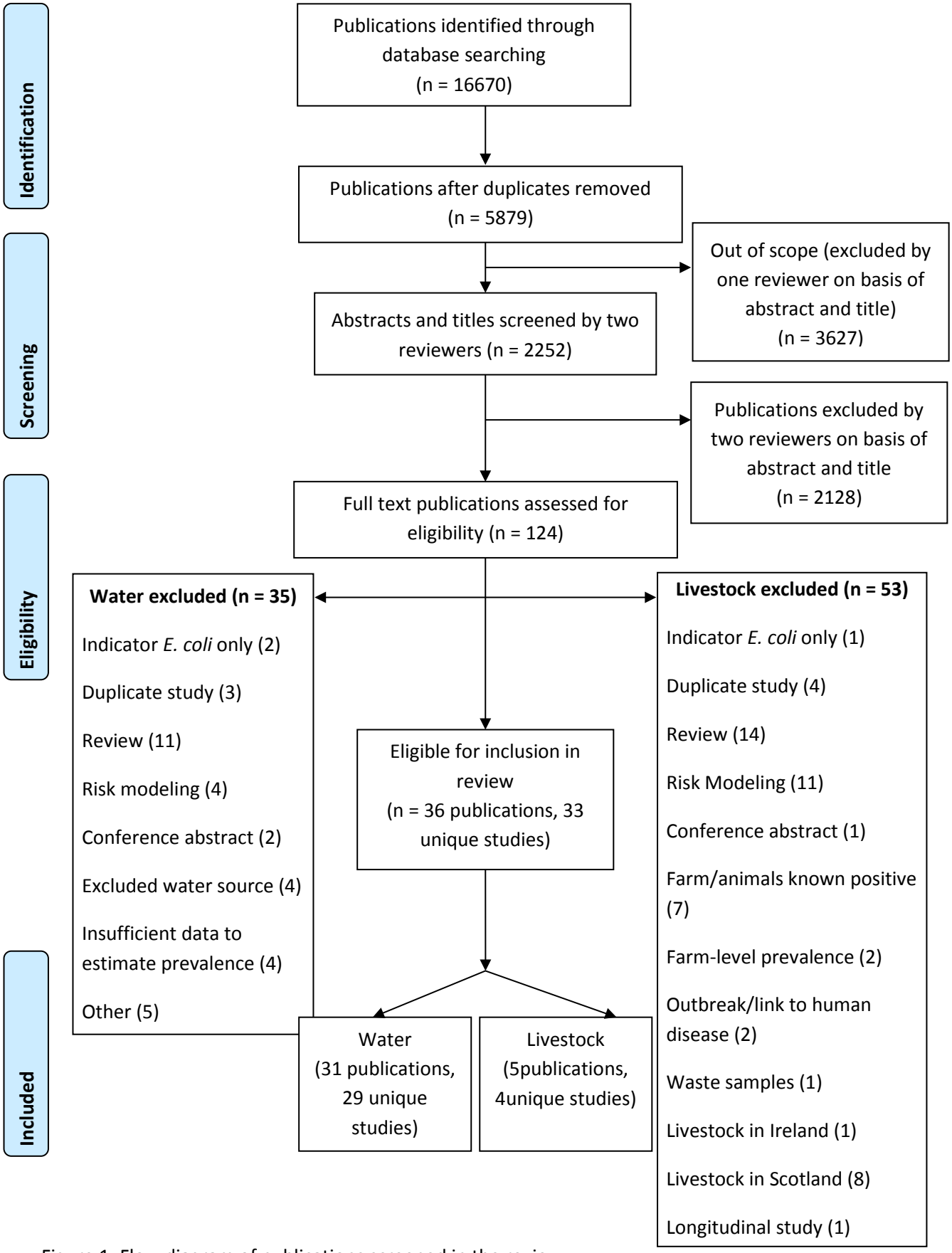


Figure 1: Flow diagram of publications screened in the review.

2.2 Water Results

Thirty-one publications reported *E. coli* O157 monitoring in water sources. Studies reporting presence/absence of *E. coli* O157 in raw water sources in 26 different locations are recorded in table 1. All raw water sources were surface water sites with exception of two aquifers. Three independent studies reported *E. coli* O157 presence/absence in public and private drinking water sources (table 2). Five publications reported bacterial counts of *E. coli* O157 in 5 raw water sources in addition to presence/absence data (table 3). Crude prevalence of *E. coli* O157 in all water sources ranged from 0 to 79%. Bacterial counts of *E. coli* O157 ranged from 10-100 to 2000 CFU/L.

2.3 Water study selection for risk assessment

As can be seen from tables 1 to 3, there is a dearth of studies that have quantified *E. coli O157* in the UK in either raw or drinking water. Just two studies addressed the relationship between the *E. coli* O157 counts and indicator *E. coli* counts (Dorner 2005 and Jenkins et al., 2007). One study that provided a fairly large dataset was the PhD thesis by Sarah Dorner (2005) based on a single watershed in Canada. This dataset contained 445 samples that had both *E. coli* O157 and indicator *E. coli* counts taken from 39 locations. In the second study, 30 samples taken from three areas of a single surface water location in Northeast Georgia, USA, were analysed for both indicator *E. coli* and *E. coli* O157 (Jenkins et al., 2007). In both datasets combined, *E. coli* O157 was detected in 24 of 479 samples (5%) and 24 of 475 samples reporting both indicator and *E. coli* O157 data.

2.4 Livestock Results

Fifteen publications (19 studies) reported pat or animal level prevalence data. These studies were conducted using longitudinal and cross-sectional designs in abattoir and farm settings with animals of mixed breed and age. Studies conducted exclusively in Scotland were excluded (Chase-Topping et al., 2007, Gunn et al., 2007, Ogden et al., 2004, Ogden et al., 2005, Omisaken et al., 2003, Pearce et al., 2004b, Shaw et al., 2004, Solecki et al., 2009, Vali et al., 2005) as was one study using a longitudinal design (Liebana et al., 2005). Of the remaining five publications, prevalence in cattle was reported by four, prevalence in sheep by three and only one publication reported prevalence in swine (table 4). Prevalence of *E. coli* O157 in the four cattle studies ranged from 4.91-12.92% and for sheep prevalence ranged from 1.35-1.97% (table 4). We did not include swine in the QMRA because of its low shedding rates and low density in England and Wales. Furthermore, the review identified only one swine prevalence study and this one study found a low prevalence of 0.61% (Milnes et al., 2008, 2009).

In terms of estimating the excretion rates of *E. coli* O157 we have adopted a two stage approach as per Dorner (2005). The first stage is to estimate the probability of whether the animal was colonised with *E. coli* O157 . The second stage is to estimate the distribution of counts in positive animals.

The results indicated that there are a reasonable number of England and Wales studies where the presence/absence of *E. coli* O157 or STEC O157 are reported. However, there were very few studies

that contained information on the concentrations of these bacteria in positive animals. Therefore, the decision was taken to estimate probability of whether animals in England and Wales were colonised with *E. coli* 0157 using studies from our literature review. However, for positive animals to model the distribution of E Coli 0157 counts in positive animals we adopted the distribution parameters reported in Dorner (2005) which are based upon the results from 65 studies from across the globe.

2.5 Conclusions of the review

The review demonstrated that there were too few studies reporting levels of *E Coli 0157* in raw waters for these to be used to in a risk assessment of *E Coli 0157 in* English and Welsh Drinking Water. Consequently the assessment of likely levels needs to be made based upon *E Coli 0157* inputs into catchments. The main input is from the faeces of cattle and sheep. For an assessment of likely inputs information is needed upon the percentage of livestock in England and Wales that are colonised with *E. coli* O157 and the distribution of counts in positive animals. From a review of the literature four studies were identified which provide robust information on the percentage of cattle that are colonised with *E. coli* O157. Three studies were identified for the percentage of sheep that are colonised with *E. coli* O157. Very few England and Wales studies were identified that contained information on the concentrations of these bacterial in positive animals. Therefore, to model the distribution of E Coli 0157 counts in positive animals we propose the distribution parameters reported in Dorner (2005) which are based upon the results from 65 studies from across the globe.

Table 1: Studies reporting presence/absence of *E. coli* O157 in raw water sources.

Reference	Country	Water Source	*E. coli* O157 positive	STEC/ other positive	Number of samples	Sample vol. collected; amount filtered/analysed; pore size	Isolation method
Ahmed et al., 2009	Australia	8 sites, 1 pond & 2 creeks		16[a]	32	5L; 500ml; 0.45-µm	QPCR
Auckenthaler et al., 2002	Switzerland	Karst spring aquifer		34[b]	55[c]	-	-
Bonetta et al., 2010	Italy	13 sites, river watershed	1	1[d]	45	-; 1L; 0.45-µm	Multiplex PCR
Cooley et al., 2007	USA	22 sites, river watershed	38[e]		584ec	-; 100ml; 0.45-µm	RT-PCR
Deschesne & Soyeaux, 2007	UK	River	0		12c	-	-
Deschesne & Soyeaux, 2007	France	Aquifer	0		10	-	-
Deschesne & Soyeaux, 2007; Astrom et al., 2007	Sweden	River	0		23c	25L; - (haemoflow or membrane filtration); -	-
Diez et al., 2009	Germany	Surface water	0[f]		161[g]	-	RT-PCR
Duris et al., 2009	USA	41 sites, multiple watersheds		39df	67	100ml; 100ml, 10ml, 1ml; 0.45-µm	Reveal, Multiplex PCR
Fincher et al., 2009	USA	5 sites, watershed	37		63	1L; 500ml; 0.45-µm	IMS, PCR
Fremaux et al., 2009	Canada	5 sites, river	0	44[h]	70	300ml; 200ml; 0.45-µm	Culture, PCR

[a] s*tx1 and/or stx*2 positive, *eae* not tested
[b] VTEC positive
[c] Includes event data, such as, heavy rainfall
[d] *eae* & *stx*1 and/or *stx*2 positive
[e] Excluding Moore swabs
[f] Testing for *E. coli* O157 in indicator bacteria positive samples only
[g] Filter samples
[h] STEC positive

Gannon et al., 2004	Canada	40 sites, river and irrigation water	27		1608	250ml; 90ml; -	IMS
Haack et al., 2009	USA	18 surface drinking water sites, multiple watersheds		8df	18	-; 100ml, 10ml, 1ml; 0.45-µm	Reveal, Multiplex PCR
Heuvelink et al., 2008	Netherlands	10 sites, surface water.	1		49	-; 1L; 0.45-µm	Immuno Diagnostic Assay System
Himathongkham et al., 2007	USA	Surface water	6		87e	-; 100ml; -	RIMS, RT-PCR, culture
Johnson et al., 2003	Canada	84 sites, river	13		1483[i]	-; 90ml; -	IMS
Jokinen et al., 2010a	Canada	4 sites, watershed	5		186	-; 500ml (3); 0.45-µm	IMS, PCR
Jokinen et al., 2010b	Canada	9 sites, watershed	8		342	-; 500ml (3); 0.45-µm	IMS, PCR
Manandhar et al., 1997	Tasmania	Surface water		3b	39	-; 100ml; 0.45-µm	Culture
Petterson et al., 2009	France	Surface drinking water source	7		13	-; 1.11L[j]; 0.2-µm	PCR
Savichtcheva et al., 2007	Japan	5 sites, surface water	6		30	3L; 3L; 0.2-µm	RT-PCR
Shelton et al., 2006	USA	19 sites, watersheds	Mean 50%	-	1303	500ml; 100ml; 0.45-µm	IM-ECL, IMS, Multiplex PCR, RTPCR
Shelton et al., 2008[k]	USA	8 sites, watershed.	27-90%			-; 0.1, 1.0, 10, 100ml (3 of each); -	IM-ECL; PCR

[i] Sample sites included storm drains and sewage treatment plants
[j] Different volumes tested
[k] Not a peer reviewed article

Smith et al., 2009	USA	5 sites, recreational lake	5[l], 37[m]	716	-; -; 0.45-µm	QPCR
Urdahl et al., 2008	Scotland	Stream with livestock access	4[n]	40[o]	Auto-sampler; 500ml (6); 0.45-µm	PCR
Wilkes et al., 2009	Canada	24 sites, 5 surface water		823	1L; 3-500ml; 0.45-µm	IMS

Table 2: Studies reporting presence/absence of *E. coli* O157 in public & private drinking water sources (treated & untreated)

Reference	Country	Water Source	*E. coli* O157 positive	Number of samples	Sample volume collected; filtered/analysed; pore size	Isolation method
Diez et al., 2009	Germany	Drinking water	0	16g/f	-	RT-PCR
Halabi et al., 2008	Austria	Public and private water supplies	0	2633	-	PCR
Schets et al., 2005	Netherlands	144 private groundwater supplies (50% no treatment)	4	144	-; 100 and 1000ml; -	IMS, RT-PCR

[l] *stx*1 positive, *eae* not tested
[m] *stx*2 positive, *eae* not tested
[n] Not including filter samples
[o] Pooled samples

Table 3: Studies reporting presence/absence and bacterial counts of *E. coli* O157 in raw water sources

Reference	Country	Water Source	No. *E. coli* O157 positive	No. of samples	Sample vol. collected; amount filtered/ analysed; pore size	Isolation method	Range of *E. coli* O157
Deschesne & Soyeaux, 2007	France	3 Rivers	19	24c	-	-	10-100 to >1000 CFU/L
Deschesne & Soyeaux, 2007	France	Reservoir	7	13c	-	-	10-100 to >1000 CFU/L
Dorner et al., 2005 & 2007	Canada	2 creeks, 3 rivers	15	449c	-; 1ml, 10ml, 50ml; -	Culture	100 to 2000 CFU/L
Heijnen & Medina, 2006	Netherlands	3 sites, surface water	2	27	-; 100ml (5x), 10ml (3x), 1ml (3x); 0.2µm	Culture-PCR	Both 4 MPN/L
Jenkins et al., 2009	USA	Pond	9	30	20L; 20L; 1-µm	Culture, PCR	0.1 to 9MPN/L

Table 4: Cross-sectional studies reporting crude prevalence of *E. coli* O157 in livestock in England, Wales and Scotland.

Livestock Type	Reference	Country	Setting	Follow-up period	Age	Breed	Sample type	Sample volume; homogenised; processed	No. STEC O157 positive animals (eae plus one or both VT genes)	No. *E. coli* O157 positive animals	No. of animals sampled	Crude prevalence *E. coli* O157 (95% CI)
1. Cattle	Chapman et al., 2001	England	1 abattoir	Apr 1997-Mar 1998	-	-	Rectal swabs	Swab; 5ml (BPW); 25µl	619	620	4800	12.92%
2. Cattle	Milnes et al., 2008, 2009	Great Britain	93 abattoirs	Jan 2003-Jan 2004	2-30 months	Beef, dairy	Rectal	1/10 dilution rectal content; 9ml (BPW); 30µl	121	134	2553	5.25%
3. Cattle	Paiba et al., 2002	Great Britain	117 abattoirs	Jan 1999-Feb 2000	<30 months	-	Rectal	1g; 9ml (BPW); 30µl	186	205	4173	4.91%
4. Cattle	Paiba et al., 2003	England & Wales	75 farms	Jun 1999-Dec 1999	All ages	Dairy,suckler, fattener	Rectal	1g; 9ml (BPW); 50µl	196	231	4663	4.95%
5. Sheep	Chapman et al., 2001	England	1 abattoir	Apr 1997-Mar 1998	-	-	Rectal swabs	Swab; 5ml (BPW); 25µl	100	100	7200	1.39%
6. Sheep	Milnes et al., 2008, 2009	Great Britain	93 abattoirs	Jan 2003-Jan 2004	<1yr	-	Rectal	1/10 dilution rectal content; 9ml (BPW); 30µl	21	38	2825	1.35%
7. Sheep	Paiba et al., 2002	Great Britain	117 abattoirs	Jan 1999-Feb 2000	<30 months	-	Rectal	1g; 9ml (BPW); 30µl	70	82	4171	1.97%
8. Swine	Milnes et al., 2008, 2009	Great Britain	93 abattoirs	Jan 2003-Jan 2004	4-36 months	-	Caecal	1/10 dilution rectal content; 9ml (BPW); 30µl	6	13	2114	0.61%

3 ELIMINATION OF *E. COLI* O157:H7 UNDER CONDITIONS RELEVANT TO TREATMENT OF PUBLIC WATER SUPPLIES

3.1 Introduction

Depending on the quality of source of water, one or more processes are required to produce drinking water that is safe and acceptable for all its intended applications, and to minimise deterioration in its quality during distribution to consumers. All processes used in water treatment can reduce the numbers of harmful organisms, regardless of whether that is their specific purpose, and constitutes the "multiple barrier" approach to safeguarding water quality.

These processes can broadly be separated into those that remove and those that inactivate pathogens. The whole of water treatment, therefore, constitutes disinfection, in which micro-organisms may be eliminated by:

- removal through physical processes (e.g. coagulation, flocculation and sedimentation, filtration and membrane filters), or
- inactivation by chemical (e.g. chlorination, ozonation) or physical treatment (e.g. UV irradiation).

Chlorination is the main process of disinfection for the majority of viral and bacterial waterborne pathogens likely to be present in sources of drinking water. For a good quality source of water, it can be the only treatment, whereas for poorer sources of water, chlorination is applied as the final treatment.

When gaseous chlorine is added to water, it reacts to produce hypochlorous acid (HOCl) which dissociates to produce the hypochlorite ion (OCl^-). Hypochlorous acid is a much stronger oxidant than hypochlorite ion, and thus is a more effective disinfectant. Below pH 4, chlorine exists in solution as the elemental chlorine. The sum of the concentrations of elemental chlorine, HOCl and OCl^- is referred to as free (available) chlorine. In practice, the pH range applied during water treatment precludes the formation of elemental chlorine, so free (available) chlorine is simply the sum of HOCl and OCl^- concentrations.

The extent of the dissociation, and therefore the proportions of HOCl and OCl^- in solution, is a function of pH and temperature. Increasing the pH promotes the formation of OCl^-, and consequently, chlorination is more effective at neutral to acidic pH than at alkaline pH. At a given pH, the amount of HOCl decreases with increasing temperature, because of increased dissociation. However, in terms of disinfection performance, this effect is compensated for by the greatly increased activity of oxidation at a higher temperature, so disinfection performance increases with temperature.

Suitable contact time must be provided, normally in purpose built tanks, to allow the necessary disinfection reactions to occur. Chlorination requirements for inactivation of an organism are usually derived in terms of a Ct product or value, where C is the chlorine concentration in milligrams (mg)

liter $(L)^{-1}$, and t is the contact time (minutes). On this basis for a given Ct, a longer exposure to lower chlorine residual has the same effect as a shorter exposure to a higher residual.

The WHO recommendations for the use of chlorine as a disinfectant stipulated a minimum free chlorine concentration of 0.5 mg L^{-1} after 30 minutes contact time at a pH of less than 8, provided that the turbidity is less than 1 NTU. This corresponds to a product of 0.5 x 30 to provide a Ct of 15 mg.min L^{-1}.

A number of factors are taken into account by water companies to ensure water receives sufficient chlorination. Several companies were found to make allowance for variation in pH and temperature, and the expected chlorine demand of the water being treated.

Chlorine demand is the reduction in chlorine concentration that occurs due to reaction between chlorine and contaminants in the water. Part of the reduction will be almost instantaneous (e.g. reaction with ammonia), part will be gradual (e.g. reaction with natural organic matter). The instantaneous demand is the difference between the initial dose of chlorine and the subsequent measurement of chlorine residual immediately downstream.

During water treatment, monitoring of the difference in the chlorine residuals across the contact tank provides a measure of chlorine demand. Typically, Ct is based on the residual chlorine concentration after the contact tank, and consequently applied Ct will be greater than target Ct. This provides a safety margin to ensure the desired degree of inactivation will be achieved.

In practice, ideal hydraulics are never observed in contact tanks. The hydraulic residence time (HRT) of each sub-volume of water passing through is not equal, but instead is characterised by some form of residence time distribution (RTD). A proportion of the water short-circuits through the tank and thus has a residence time less than the HRT; while other sub-volumes recirculate or get caught in quiescent zones and thus have residence times greater than the HRT.

To correct for the variation in real residence time, a common approach is for water companies to apply a value for time (t_x) that corresponds to the period of time for a specified proportion of water to pass rapidly through the tank. Typically, Ct is based on the assumption that 90 per cent of the flow through the contact tank has the required exposure period, and 10 per cent (t_{10}) has received less treatment. A value for T can be obtained from tracer tests on specific contact tanks.

The susceptibility of *E. coli* has been widely studied and is known to be readily inactivated after a short period of exposure to low concentrations of free (available) chlorine. Thus, considerable inactivation of this organism is achieved by Cts that are well below the WHO guideline value of 15 mg.min L^{-1}. However, a number of factors are known to impair chlorination and these may reduce its efficacy in practice. These factors relate to the intrinsic condition of the organism, the characteristics of the water being treated and the design and operation of the chlorination process.

The purpose of this review is to determine whether *E. coli* O157 behaves similarly to typical *E. coli* during chlorination, and determine if there are any factors likely to interfere with inactivation during water treatment. This would permit the significance of the studies on disinfection to be assessed in relation to chlorination practice at a water treatment works.

3.2 Literature review

The assessment was conducted entirely as a review of the published literature. A systematic search was undertaken using key words representing water treatment, disinfection and the organism (Table 5). The primary focus was on chlorination, but the scope of the review was extended to include other treatment processes.

Table 5: Keywords used for the literature search.

Category	Search terms
Water Treatment	Removal, Clarification, Filtration
Disinfection	Inactivation, Reduction, Chlorine, Chlorination, Ultraviolet
Organism	*E. coli* O157, STEC, Pathogenic *E. coli*

The searches were conducted mainly in two databases, Aqualine (Cambridge Scientific Abstracts) and PubMed (US National Library of Medicine National Institutes of Health Search). Additionally, WRc maintains a database for UKWIR / DWI which compiles periodic reviews of topics of concern from micro-organisms in drinking water. The reviews over last five years (2006 to 2011) were searched for references associated with *E. coli* O157 and water treatment.

All references were selected that reported inactivation of *E. coli* O157 by free (available) chlorine. Of most relevance were those studies that had examined inactivation in the context of water treatment, although some studies associated with food hygiene were included where they contained data on chlorination.

3.3 Disinfection of *E. coli* O157

3.3.1 Removal

At treatment works with conventional, multiple-barrier treatment (coagulation, flocculation and sedimentation and rapid gravity filtration) before final chlorination, it would be expected to achieve at least a three $\log_{10}$ reduction in bacteria such as *E. coli* (Pitchers, 2010). Bacteria can be removed by binding to flocs formed during the coagulation processes or become retained on filter media.

Only a single study has investigated the reduction of *E. coli* O157 by physical treatment. A pilot-scale system used by Harrington (Harrington et al., 2003) demonstrated removal of around 0.4 to 0.5-$\log_{10}$ during filtration after coagulation and sedimentation, and was not affected by the filter loading rate, between 23 and 90 m h^{-1}, and type of filter medium. A greater than 1-$\log_{10}$ improvement in removal was obtained when coagulation preceding filtration was performed at pH of 5.7 rather than pH 7.0.

3.4 Inactivation

3.4.1 Free (available) chlorine

Seven studies were identified from the literature that had examined the inactivation of *E. coli* O157:H7 by free (available) chlorine (see Table S5 in the appendix). However, only five were conducted as proper inactivation studies that were directly relevant to water treatment, although the findings from the other studies were included to provide corroborating information.

All the reported studies have shown that *E. coli* O157:H7 is readily inactivated by free (available) chlorine, and show a response that is similar to other strains. Over 4-$\log_{10}$ reduction in numbers of viable cells was achieved with Cts below 1.0 mg.min L^{-1}.The inclusion of strains recently isolated from the environment allowed for an expected increase in resistance to chlorination compared to culture collection strains. Some strains of environmental origin exhibited slighter greater resistance to chlorination but the effect was not consistent for all strains. However, in these studies strains were cultured under nutrient rich conditions before chlorination which could increase their susceptibility to inactivation.

The experiments were also conducted under ideal conditions whereby no substances were present that would have interfered with disinfection. Consequently, extrapolation of an effective Ct to conditions encountered in practice would need to take account of the various factors known to impact on the efficacy of chlorination.

No systematic evaluation has been undertaken to examine the influence of temperature and hydrogen ion concentration (pH) on chlorination. The efficacy of chlorination decreases with lowering temperature. However, one study carried out at 5 °C, which is more representative of a worst case situation, found good inactivation of *E. coli* O157:H7. This provides reassurance that temperature would not have a significant impact on the effectiveness of chlorination applied to the range of source waters encountered in England and Wales. The studies have been conducted at a pH around neutral. Water for treatment in England and Wales could be as high as pH 8.5 in certain circumstances, which would be less favourable for inactivation.

The derivation of the Ct values for inactivation of *E. coli* O157:H7 has assumed that chlorine concentration remains constant during the course of the contact time. This may be true for laboratory experiments in demand free systems, but it is not the case at water treatment works, where the demand of the system causes a gradual decline in the active concentration of the disinfectant.

Attachment of cells can provide protection from chlorination. It is well known that cells in biofilms show greater resistance to inactivation. This situation is not expected to occur during water treatment, as the conditions would not permit *E. coli* O157:H7 to colonise biofilms. Cells attached to particles or incorporated into flocs can also be more resistant to inactivation (Camper et al., 1986). However, where particles likely to contain *E. coli* O157:H7 are present in a source of water, the processes before chlorination will substantially reduce their number such that they would exert minimal impact on the effectiveness of chlorination.

Many bacteria are capable of developing into a viable but non-culturable condition (VBNC) in response to adverse environmental conditions. A cell is considered to be in a VBNC state if it remains metabolically active but is incapable of multiplying in numbers to produce a colony on a culture medium known to support growth of uninjured cells. The mechanism giving rise to this condition is not properly understood, but it has been linked to the inability of cells to adapt to a metabolic imbalance when stressed cells are presented with nutrients, which causes free radical production to damage cell integrity.

Several studies have shown that *E. coli* O157 becomes VBNC during prolonged storage in water devoid of nutrients. Cells in this condition are considered to be more robust and thus have an increased resistance to chlorination. Lisle *et al*. (1999) reported that prolonged exposure of cells to starvation increased their resistance to chlorination. The effect occurred at a very low Ct of 0.25 mg.min L^{-1}. It is unlikely that stressed cells would withstand inactivation at higher Cts

For stressed cells to represent a public health threat they would have to recovery their viability and be capable of causing infection. Kolling and Matthews (Kolling and Matthews, 2001) exposed two strains of stationary-phase *E. coli* O157:H7 cells, starved for 7 days in water, to free (available) chlorine (50 mg L^{-1}) for 30 seconds. No colonies developed on TSA medium, or if supplemented with sodium pyruvate, and mT7 agar, showing complete loss of culturability. Viable cells were observed by Baclight staining, indicating that some of the cells were still intact and metabolically active. Additionally, passage of disinfected treated cells through the mouse gastrointestinal tract did not restore culturability, based on examination of faecal material, and examination of their kidneys did not reveal any significant differences to those from unexposed mice.

3.4.2 Ultraviolet irradiation

E. coli O157:H7 appears to have similar susceptibility to inactivation as other types of *E. coli*. Hijnen et al., 2006, reviewed the available literature to obtain data that permitted calculation of a microbial inactivation credit (MIC) for UV disinfection of *E. coli* O157:H7 in drinking water treatment. A 4-$\log_{10}$ reduction in cell number was achieved by exposure to 19 mJ cm^{-2} (Hijnen et al., 2006), which is lower than the UV dose normally used in water treatment of between 25 and 40 mJ cm^{-2}. The derivation of the MIC for UV disinfection included a correction to account for the differences in susceptibility to inactivation of *E. coli* O157:H7 determined under laboratory conditions and that required in practice. The experimental studies indicated a requirement to double the dose derived from the laboratory investigations to achieve a similar level of inactivation under conditions encountered during water treatment.

Organisms have the ability to repair the damage caused by UV irradiation, and over time cells can recover their viability. Two types of repair have been described: dark repair and photo-reactivation. Dark-repair does not require light and has been demonstrated in almost all bacteria. Photo-reactivation occurs in conditions of prolonged exposure to (visible) light, and although *E. coli* has this recovery mechanism, it cannot occur in a water supply system as light is absent. Also, *E. coli* may not always be capable of recovery. Zimmer-Thomas *et al*. (Zimmer-Thomas et al., 2007), demonstrated that photo-reactivation of *E. coli* did not occur after MP-lamps, an observation also supported by Oguma *et al*. (Oguma et al., 2002).

3.5 Application to water treatment

Water companies have a disinfection policy that prescribes Ct across their range of treatment works depending on the source. A Ct of 5 mg.min L^{-1} typically would be used for groundwater, with 15 mg.min L^{-1} for reasonable quality surface water, and 30 mg.min L^{-1} for poor quality surface water.

WRc obtained data from water companies on their policy chlorination (Table 6). The Ct values were found to vary, although most had adopted the guideline value of 15 mg.min L^{-1} proposed by WHO. This could be adjusted on the basis of the perceived risk, and for one company varied between 5 and 60 mg.min L^{-1} depending on the quality of the source water.

Table 6: Chlorination policy for water companies

A Ct of 60 mg.min L^{-1}, sometimes without pH compensation.
Ct between 15 and 60 mg.min L^{-1} depending on water quality
A contact time of 30 minutes at 0.5 mg L^{-1} with pH factored in using t_{10}
A contact time of 20 minutes at 0.5 mg L^{-1} with pH factored in using t_5
A Ct of 20 mg.min L^{-1} with an additional 0.3 mg L^{-1} for surface water and a Ct of 5 mg.min L^{-1} with an additional 0.3 mg L^{-1} for ground water.
Cts of 30 mg.min L^{-1} for surface water and 15 mg.min L^{-1} for ground water.

Typically, higher Cts are applied for surface water compared to ground water. Where chlorination was preceded by an additional inactivation process, such as UV irradiation, a lower Ct could be applied to compensate for the additional upstream inactivation. Therefore, taking 5 and 15 mg.min L^{-1} would be representative of chlorination for ground and surface water sources respectively.

3.6 Conclusions

- Under ideal conditions, *E. coli* O157:H7 is very susceptible to inactivation by free (available) chlorine under conditions applied during water treatment, exhibiting a response that is no different to non-pathogenic strains of *E. coli*.
- Chlorination during water treatment is conducted with Cts that are capable of inactivating considerable numbers of *E. coli* O157:H7, under all conditions typically encountered in practice.
- Certain *E. coli* O157:H7 strains appear to possess an inherent greater resistance to inactivation, but not sufficiently so that they would survive chlorination during water treatment.
- Where cells of *E. coli* O157:H7 have been exposed to adverse conditions, they can develop a more robust cell type that permits greater resistance to inactivation. However, this occurs at concentrations of free (available) chlorine that are considerably lower than used during chlorination, and so this mechanism would not interfere with elimination of the organism during water treatment.

4 Public water supply assessment: Site Visits

4.1 Introduction

Visits were undertaken to selected water companies to identify a suitable number of catchment to tap water supply systems that could be included in the risk assessment for public supplies. For the purpose of the risk assessment, cattle and sheep were assumed to be the significant source of *E. coli* O157 in a catchment. To minimise potential interferences in the analysis, catchments were excluded that contained significant faecal contamination of human origin.

4.2 Catchment types

In collaboration with each water company, at least three sites were identified where faecal contamination was predominantly from cattle and sheep grazing the surrounding land. Where more than three sites were identified a random selection was performed. Various sources of water were selected that included discrete bodies of surface water, such as upland rivers and reservoirs as well as sources of groundwater that were under the influence of surface water containing faecal contamination originating from livestock (Table 7).

For each source of water, data was obtained on the numbers of *E. coli* monitored at the abstraction point for the corresponding water treatment works. A period, representing the last five years, was selected to indicate the variability, particularly seasonal effects, in the numbers of these bacteria in the source water.

4.3 Water treatment practice

For each abstraction point in the catchment, information was obtained on corresponding water treatment practice (Table 7). This comprised all the individual processes to establish the overall elimination capacity of each treatment works. It was recognised that physical processes, such as clarification (coagulation, flocculation and sedimentation) and filtration would remove significant numbers of *E. coli* O157 in addition to chlorination and other inactivation processes such as UV irradiation and ozonation (See review in Section 3).

Table 7: Description of sites included in the risk assessment

Site	Site Code	Water Source	Catchment	Treatment
1	A1	Surface (Direct abstraction from a lowland stream)	Moderate dairy and sheep farming, but extensive slurry and dung spreading throughout the catchment. Human faecal input from a limited number of septic	Coagulation – Flocculation – Sedimentation - Rapid Gravity Filtration - Chlorination

			tanks.	
2	A2	Surface (Stream fed upland reservoir)	Sparse dairy and sheep farming, but extensive slurry and dung spreading throughout the catchment. Significant bird roosting by the reservoir. Average retention time of 133 days in the reservoir.	Coagulation – Flocculation – Sedimentation - Rapid Gravity Filtration - Chlorination
3	A3	Surface (Stream fed upland reservoir)	Intensive dairy and sheep farming, with extensive slurry spreading throughout the catchment. Significant bird roosting by the reservoir. Average retention time of 129 days in the reservoir.	Coagulation – Flocculation – Sedimentation - Rapid Gravity Filtration - Chlorination
4	C1	Surface (River and reservoir)	Intensive calf and cattle stocking throughout the catchment, other livestock is sparse and scattered.	Coagulation – Dissolved air flotation – Rapid Gravity Filtration (sand) – Manganese removal - Chlorination
5	C2	Surface (Lowland river)	Intensive sheep farming and sparse cattle grazing throughout both catchments. Also, manure applications to arable land.	Pre-ozone - Coagulation – Dissolved air flotation – Rapid Gravity Filtration (sand) – Ozone – Post filtration absorption (GAC) - Chlorination
6	C3	Surface (Spring)	Upland sheep farming with little cattle grazing.	Coagulation – Floc blanket clarification – Rapid Gravity Filtration (sand) - Chlorination
7	B1	Surface (Upland reservoir)	Sparse cattle within the wider catchment. Acid loams and peat soils surround the catchment. Occasional high levels of colour seen in the raw water.	UV irradiation - Chlorination
8	B2	Surface (Upland stream)	Sparse numbers of cattle within the catchment. Acid soils and blanket bog.	UV irradiation - Chlorination
9	B3	Surface (Lowland River)	There are large number of beef cattle and small numbers of dairy cattle.	Coagulation – Flocculation – Rapid Gravity Filtration – Chlorination
10	B4	Surface (River and reservoir)	Beef cattle and intensive dairy farming within the lower reaches of the catchment.	Coagulation – Dissolved air flotation – Rapid Gravity Filtration (sand) - Chlorination
11	D1	Surface (Reservoir)	Livestock grazing in the catchment. Less than 7 days retention time in the reservoir.	Coagulation – Dissolved air flotation – Pressure filtration (sand) – Absorption (GAC) – Chlorination

12	D2	Surface (Reservoir)	Livestock grazing in the catchment. Greater than 7 days retention time in the reservoir.	Coagulation – Dissolved air flotation – Pressure filtration (sand) – Absorption (GAC) - Chlorination
13	D3	Surface (Reservoir)	Livestock grazing in the catchment. Nominal 20 days retention time in the reservoir.	Pre-chlorine - Coagulation – Dissolved air flotation – Rapid gravity (sand) - Chlorination

4.4 Reliability of chlorination

The reliability of chlorination was determined from disinfection failures that were reported to DWI, and were published in conjunction with their Annual Report. The frequency of impaired chlorination was determined for the last two year's reporting period (Table 8). This review excluded detection of *E. coli* or coliforms at treatment works where chlorination was being carried out satisfactorily.

The frequency of occurrence was around one incident per month over both years. The duration of most incidents was less than 24 hours, although there were some notable exceptions. However, the duration would represent the time taken to restore water supply rather than detection of the actual incident. Consequently, the time period does not represent the period corresponding to water supplied without chlorination.

For the purposes of the risk assessment, the scenario for modelling risks from failure in chlorination was one incident per month of 24 hours and would represent an extreme event.

Table 8: Reported incidents of loss of disinfection over one year.

Date	Duration (hours)	Population served by the supply	Cause
29 Jan 2010	24	100 000	Loss of disinfection.
04 Feb 2010	6	8 000	Temporary loss of power leading to un-disinfected water leaving site.
11 Mar 2010	144	150 000	Loss of disinfection.
21 Mar 2010	1	1 500 000	Loss of disinfection.
03 Apr 2010	12	45 228	Loss of disinfection.
10 May 2010	4	39 165	Loss of disinfection.
21 May 2010	3	111 627	Compromised disinfection.
07 Jun 2010	4	380 000	Loss of disinfection.
24 Sep 2010	96	15 490	Loss of chlorine in final water
12 Dec 2010	1	93 000	Loss of disinfection.
22 Dec 2010	8	98 000	Loss of disinfection.
08 Jan 2009	6	180 000	Loss of lime dosing and inadequate disinfection following power failure.
29 Jan 2009	2	56 600	Inadequate disinfection due to plant failure.
08 Mar 2009	3	180 000	Inadequate disinfection due to power failure.
20 May 2009	3	180 000	The treated water continued to be dechlorinated despite low chlorine residuals.
27 May 2009	1	30 000	Inadequate disinfection due to change in water treatment.
28 Jun 2009	24	55 000	Inadequate disinfection due to borehole pumps continuing to operate following works shut down.
18 Jul 2009	15	620 302	Loss of coagulation and failure of disinfection.

16 Aug 2009	48	180 000	Inadequate disinfection due to power failure.
26 Aug 2009	48	2 000 000	Failure of disinfection.
19 Oct 2009	3	44 150	Loss of disinfection due to plant failure.
19 Oct 2009	24	33 059	Inadequate disinfection.

4.5 Integrity of the distribution system

As *E. coli* is considered to be exclusively of faecal origin, its detection in a distribution system indicates a breach in integrity that may be associated with the presence of harmful organisms. During the last full reporting period (2010), *E. coli* was detected on 17 occasions across around 4 400 reservoirs in England and Wales (Table 9). Correspondingly, during the same period, about 40 mains bursts were reported, although there was no evidence to indicate that these resulted in ingress of faecal contamination (Table 10).

The risk specifically from *E. coli* O157 in a distribution system depends on a breach in the integrity of water mains which permitted ingress of faecal contamination originating from livestock. During the site visits, most water companies were not aware of any such incidents in their water supply networks.

One water company had reported an incident where it was noted that a local farmer had moved a manure pile to within a few meters of a service reservoir. However, contamination appeared to be restricted to the tap used to collect the samples for regulatory monitoring rather than as a consequence of ingress into the service reservoir.

Table 9: Reported coliform detection at service reservoirs

Region and company	Number of service reservoirs	Number of water supply zones	Length of water mains (Km)	Number of non-compliance at service reservoirs	
				Coliforms	*E. coli*
Central					
DCWW	25	83	27 219	2	0
STW	491	210	46 573	7	1
SSW	35	19	5 926	3	0
Eastern					
AW	370	164	37 001	8	2
CW	33	10	2 316	0	0
ESW	109	53	8 637	8	0
IWN	0	4	907	n/a	n/a
VW (E)	7	4	15	0	0
Northern					
DVW	2	5	1 848	0	0
HW	6	3	597	1	0
NW	215	75	17 061	14	4
PWN	0	1	1.2	n/a	n/a
UU	378	241	42 391	18	1

YW	359	76	31 062	17	1
Southern					
PW	31	13	3 266	0	0
SEW	224	90	14 177	14	0
SW	205	84	13 814	3	0
SSEW	0	1	9	0	0
VW (SE)	13	6	1 109	0	0
Thames					
IWN	0	2	15	0	0
SSEW	0	4	9	0	0
SESW	32	20	3 436	0	0
TW	376	237	31 453	17	4
VW (C)	134	70	14 500	1	0
Western					
BWHW	20	10	2 792	0	0
BW	165	52	6 663	0	0
CHW	1	1	30	0	0
SWW	284	32	15 000	10	3
SSEW	0	1	9	0	0
VW (Project)	6	1	98	0	0
WxW	298	91	11 000	6	0
Wales					
ALW	0	1	0	0	0
DVW	29	13	1 848	1	0
DCWW	453	77	27 219	10	1
STW	56	9	46 573	2	0

Table 10: Reported breaches in the integrity of water mains

Date	Incident	Population affected (estimated)
06 Jan 2010	Brown discolouration: due to burst on private supply.	34,250
11 Jan 2010	Loss of supplies /poor pressure due to burst main.	13,953
13 Jan 2010	Brown discolouration due to a burst main.	62,000
20 Jan 2010	Brown discolouration due to a burst main.	
21 Jan 2010	Burst main due to damage by third party.	245
21 Jan 2010	Sulphurous taste and odour due to valve operations following burst main.	6,069
21 Jan 2010	Loss of supplies /poor pressure due to burst main.	8,775
13 Feb 2010	Burst main	20,886
16 Feb 2010	Loss of supplies due to third party damage to main.	2,000
11 Mar 2010	Brown discolouration due to a burst main.	200,000
15 Mar 2010		120,000
02 Apr 2010	Discolouration due to burst main.	58,000
13 Apr 2010	Discolouration due to burst main.	6,250
17 Apr 2010	Issue of Boil Notice due to burst main.	78

20 Apr 2010	Brown discolouration due to planned work.	59,350
10 May 2010	Cross connect ion with a private supply.	300
25 May 2010	Brown discolouration due to a burst main.	12,785
01 Jun 2010	Microbiological contamination due to cross connection with rainwater harvesting system.	12
10 Jun 2010	Brown discolouration due to a burst main.	10,140
05 Jul 2010	Brown discolouration due to a burst main.	250
08 Jul 2010	Misconnection of a property to a sewer.	3
09 Jul 2010	Burst main due to planned work.	11,097
10 Jul 2010	Brown discolouration due to a burst main.	6,229
06 Aug 2010	Discolouration due to burst main.	23,893
11 Aug 2010	Burst main	2,022
12 Aug 2010	Loss of supplies/poor pressure.	187,375
17 Aug 2010	Microbiological contamination following burst main.	2,250
25 Aug 2010	Discolouration due to burst main.	18
25 Aug 2010	Third party damage to main.	50,000
19 Sep 2010	Brown discolouration due to a burst main.	12 700
26 Sep 2010	Burst main and risk of sewage ingress.	155
15 Oct 2010	Loss of supplies due to a burst main.	39,150
28 Oct 2010	Loss of supplies due to a burst main.	6 201
10 Nov 2010	Media interest about a burst main	23,750
11 Nov 2010	Brown discolouration due to a burst main.	6,643
08 Dec 2010	Loss of supplies/poor pressure due to burst main.	
17 Dec 2010	Brown discolouration due to burst main.	28,205
29 Dec 2010	Brown discolouration due to a burst main	16 900

4.6 Conclusions

Sources of water abstracted for producing drinking water face a persistent challenge from *E. coli* O157. Surface sources of water are more exposed to livestock contamination compared with groundwater sources. Consequently, the risk assessment in Section 6 has been based on catchments with livestock grazing, where their faecal contamination acts as a source of *E. coli* O157. Such supplies receive robust water treatment, but allowance was made for a failure in chlorination to take account of a deficiency in treatment. Whilst a period of 24 hours, without chlorination was used in the risk assessment, this value was considered very much a worst case situation, since most action would be taken to minimise risks to public health in a much shorter time frame.

The distribution system can be intermittently challenged by faecal contamination, and E. coli was occasionally detected in the water supply. However, no evidence was available to determine the origin of the faecal contamination, and so it could not be attributed to livestock sources. Whilst water companies considered that a theoretical risk existed, none had any knowledge of such incidents.

5 Private Water Supplies in England

A random sample of households served by private water supplies in the counties of Norfolk, Suffolk and Herefordshire were contacted by post to request their participation in a prospective cohort study. A site visit to the 270 consenting households was undertaken between January 2008 and December 2010 during which a questionnaire about treatment and a risk characterisation survey was completed.

Questions about the source of the supply, treatment method and treatment maintenance were completed by the householder as part of the Household Questionnaire. A risk assessment survey was also completed by the researcher and the householder for each supply, this assessment was based upon the key questions utilised in the Scottish Private Water Supplies Technical Manual (Scottish Executive, 2006). A further 30 questions were excluded from the Scottish survey as they were based on results of previous risk assessments, suited for larger supplies and/or were otherwise not considered applicable; four questions were also added to the survey (24. 25. 33 and 34).

5.1 Household Questionnaire Results

The majority of supplies included in the cohort study served one household 75% (197/264) with just 6% (16/264) serving more than 4 households. Fifty-seven percent of households were supplied by a borehole (154/270), 13% (35/270) by a spring, 29% (78/270) by a well and 1% (3/270) by other surface water.

Table 11 describes the number of households using each treatment method (filtration, chlorination or ultraviolet), those reporting use of at least one form of treatment, and whether the householder kept a record of treatment maintenance. Only 40% (106/267) of households reported using at least one treatment method (UV, Chlorination, Filtration) and only 59% (53/106) of these householders kept a record of treatment maintenance. Compared to other supply sources, households supplied by boreholes reported the lowest proportion of treatment use (32% (49/153)).

5.2 Risk Assessment Results

Typically, individual supplies are allocated a score based on responses to the risk characterisation (yes/no/don't know) and a hazard assessment (based on likelihood and severity values). The results presented in table 12 focus on the risk characterisation component and are summarised in terms of frequency of responses. The results are presented in this way largely because supplies included in the survey often served just one household, and for some questions this led to a large proportion of 'don't know' responses.

Items occurring with high frequency included 73% (188/257)of supplies with evidence of wildlife/domestic animals around source, 91% (234/257) of supplies with unsewered human sanitation (including septic tanks), 41% (33/81) of springs/wells with no stock proof fence at a

minimum of 4 metres around the source, 65% (11/17)of springs where the inlet pipe is not fitted with course filter or screen, 68% (105/155)of supplies where no maintenance (including chlorination) has been undertaken in the previous 12 months and 75% (86/115) of supplies where the header tank (if present) had not been cleaned in the last 12 months.

Table 11: Details of Treatment by Supply Type from the Household Questionnaire (n=270)

Source of Supply	Reported using Treatment Method			Reported using at least one of the three treatments (UV, Filter, Chlorination)?				Reported keeping a record of treatment maintenance?				
	UV	Particle Filter	Chlorination	Yes	No	Don't know	% Yes (of yes/no responses)	Yes	No	Don't Know	% Yes (of yes/no responses)	Total Supplies
Borehole	14	46	1	49	104	1	32.03	27	16	6	62.79	154
Spring	14	14	0	16	19	0	45.71	4	6	6	40.00	35
Well	26	27	1	39	37	2	51.32	21	15	3	58.33	78
Other surface water	1	2	1	2	1	0	66.67	1	0	1	100.00	3
Total supplies	55	89	3	106	161	3	39.70	53	37	16	58.89	270

Table 12: Frequency of Responses to the Risk Assessment (n=270).

	General Site Survey	Yes	No	Don't Know	Not Applicable	Missing	% 'Yes' (of yes/no responses)
1	Evidence/history of poor drainage causing stagnant/standing water (springs/boreholes/wells only)	4	236	26	3	1	1.67 (4/240)
2	History of livestock production (rearing, housing, grazing – including poultry)	100	165	5	0	0	37.74 (100/265)
3	Evidence of wildlife/domestic animals	188	69	12	0	1	73.15 (188/257)
4	Soil cultivation with wastewater irrigation or sludge/slurry/manure application	12	239	19	0	0	4.78 (12/251)
5	Surface run-off from agricultural activity diverted to flow into the source/supply	1	253	16	0	0	0.39 (1/254)
6	Farm wastes and/or silage stored on the ground (not in tanks or containers)	15	240	15	0	0	5.88 (15/255)
7	Remediation of land using sludge or slurry	4	248	17	0	1	1.59 (4/252)
8	Unsewered human sanitation including septic tanks, pit latrines, soakaways	234	23	12	0	1	91.05 (234/257)
9	Sewage pipes, mains or domestic (e.g. leading to/from septic tank)	74	123	72	0	1	37.56 (74/197)
10	Sewage effluent lagoons	2	257	10	0	1	0.77 (2/259)
11	Sewage effluent discharge to adjacent watercourse (where present)	16	235	18	0	1	6.37 (16/251)
12	Below ground chamber not watertight? (boreholes only)	5	30	113	116	6	14.29 (5/35)
13	Borehole lining (casing) does not extend at least 150mm above level of floor? (boreholes)	2	41	110	116	1	4.65 (2/43)
14	Watertight lining cap not fitted? (boreholes)	3	33	117	116	1	8.33 (3/36)
	Supply Survey						
15	No stock proof fence (to BS1722 or equivalent) at a minimum of 4 metres around the source? (spring/wells)	33	48	26	157	6	40.74 (33/81)
16	No suitable barrier present to prevent ingress of surface flows into the chamber (e.g. cut-off ditch lined with impermeable material, steep incline/decline such as embankments, appropriate walls, etc). (springs/boreholes/wells only)	28	133	104	0	5	17.39 (28/161)
17	No concrete apron, a minimum of 1200mm, sloping away from the well and in good repair? (wells)	18	32	24	192	4	36.00 (18/50)
18	The top of the chamber/well is not 150mm above ground level/the apron? (wells/boreholes)	8	84	127	38	13	8.70 (8/92)
19	No reinforced pre-cast concrete cover slab, or equivalent, in satisfactory condition with a watertight, vermin-proof inspection cover present to BS497 (lockable steel type or equivalent) with or without ventilation? (wells/springs(if present)/boreholes)	22	156	70	9	13	12.36 (22/178)
20	Overflow/washout pipe not fitted with vermin proof cap? (springs)	10	7	18	235	0	58.82
21	Inlet pipe not fitted with course filter or screen? (springs)	11	6	17	235	1	64.71 (11/17)
22	The chamber/construction is in an unsatisfactory state-of-repair? (wells/springs/boreholes)	8	78	170	3	11	9.30 (8/86)
23	No maintenance (including chlorination) has been undertaken in the previous 12 months?	105	50	114	0	1	67.74 (105/155)
	Distribution Network						
24#	Buried water distribution pipes are exposed to the surface (i.e. visible)?	2	193	72	0	3	1.03 (2/195)
25#	Cracks/pits/holes are present on exposed pipes?	0	160	103	0	7	0.00 (0/160)
26	Supply network constructed from material liable to fracture, e.g. asbestos-concrete, clay etc.?	2	41	223	0	4	4.65 (2/43)
27	Junctions present in the supply network, particularly supply animal watering systems (drinking troughs or irrigation), have no back-siphon protection?	9	77	166	16	2	10.47 (9/86)

	Water Storage / Holding Tanks						
28	If present, intermediate tanks (e.g. collection chambers, holding tanks, break-pressure tanks) are not adequately protected? (according to 12-19 above)	10	88	105	66	1	10.20 (10/98)
29	If present, header tank within the property(s) does not have a vermin proof cover?	8	108	117	36	1	6.90 (8/116)
30	If present, header tank has not been cleaned in the last 12 months?	86	29	118	36	1	74.78 (86/115)
	Treatment System						
31	Any point of entry/point of use treatment equipment has not been serviced in accordance with the manufacturer's instructions in the last 12 months?	8	84	12	164	2	8.70 (8/92)
32	If present, ultraviolet (UV) lamps are not operating?	5	48	2	215	0	9.43 (5/53)
33#	If present, chlorinator is not functioning correctly?	0	2	1	268	0	0.00 (0/2)
34#	If present, filter is not functioning correctly?	2	74	12	121	1	2.63 (2/76)
35	Is there a noticeable change in the level and flow of water throughout the year?	27	239	2	0	2	10.15 (27/266)
36	Is there a noticeable change in the appearance of the water (colour, turbidity – cloudiness) after heavy rainfall or snow melt?	66	201	2	0	1	24.72 (66/267)

Questions 24, 25, 33 and 34 were added and do not form part of the Scottish PWS Risk Assessment.

5.3 Conclusions

The results of the risk characterisation demonstrate the vulnerability of supplies; it is therefore surprising that only 40% of supplies included in the cohort study employed an adequate form of treatment. The majority of households included in this study (75%) serve just one household and the statutory requirements for risk assessment and monitoring outlined in The Private Water Supplies Regulations 2009 (Her Majesty's Government, 2009) do not apply to this potentially vulnerable group.

When interpreting results, it should be taken into consideration that households volunteered to participate and therefore results may not reflect the characteristics of all private supplies in England and Wales. The treatment methods used and the vulnerability of supplies may differ between households volunteering to participate and those not volunteering to participate.

6 Quantitative Risk Assessment

6.1 Introduction

In this section we report on the results of a quantitative microbial risk assessment carried to assess the risk of infection in due to Shiga toxin positive *E. coli* O157. In undertaking this assessment we have followed the general strategy towards QMRA of drinking water as set out in the reports of the MicroRisk project (Smeets, 2006).

6.2 Methods

6.2.1 Modelling software packages

For all MonteCarlo analyses we used @Risk 5.7^{TM} (Palisade). Models were run for 10,000 iterations.

6.2.2 Selection of study sites

Altogether 13 sites were randomly chosen from four different English water companies. These sites were chosen randomly to give a representative sample of water utilities in the Country. Detailed descriptions of these 13 sites are given in Table 7 (Section 4.3).

6.2.3 General assumptions

The key assumption in this study is that *E. coli* O157 has the same survival characteristics as indicator *E. coli*, as has been shown above. Consequently, and unlike the situation with other pathogens, we can assume that the ratio between *E. coli* O157 and indicator *E. coli* in the raw and treated waters will be the same as in fresh manure of mammals in the catchment area.

6.2.4 Estimating *E. coli* O157 concentrations in raw water.

The datasets based on a single watershed in Canada (Dorner 2005) and a surface water source in the USA (Jenkins et al., 2007) identified in the review (see Section 2)) were extracted. When combined, this dataset contained a total of 475 samples that had both *E. coli* O157 and indicator *E. coli* counts taken from 40 locations. *E. coli* O157 was detected in 24 (5%) samples. The median count of *E. coli* O157 in positive only samples was 25 cfu/100ml (10th percentile = 0.05 and 90th percentile=100).

We estimated the relationship between *E. coli* O157 and indicator *E. coli* using a predictive model, developed within STATA™, from the dataset (Dorner 2005 and Jenkins et al., 2007). We used censored linear regression of the log transformed *E. coli* O157 counts with log indicator *E. coli* count +1 as the predictor variable. The estimated variance of error was then calculated using the fitstat command. The estimated regression equation is given below in Table 13. The estimated variance of error was 5.844.

Table 13: Regression equation predicting *E. coli* O157 counts from indicator *E. coli* counts/100ml

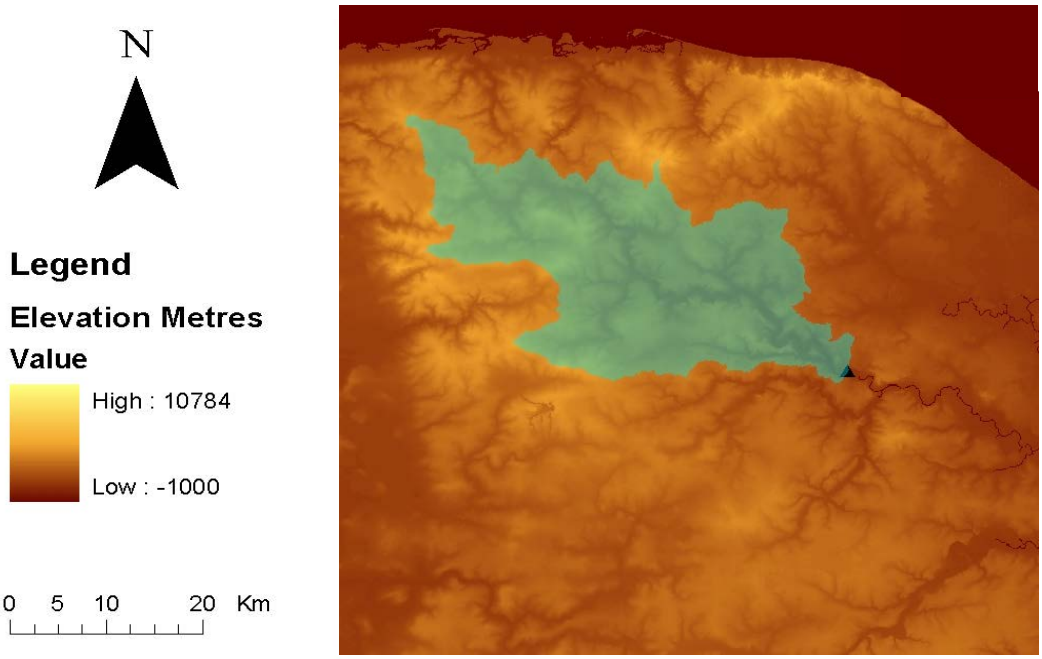
log0157	Coef.	Std. Err.	P>t	[95% Conf.	Interval]
logecoli1	0.595	0.336	0.078	-0.066	1.256
_cons	-5.421	1.291	0	-7.958	-2.884

The variance of error in the model was substantial and consequently this model was not further used. Instead we developed estimates of the ratio O157:indicator *E. coli* ratios for each study site, using Livestock numbers for each catchment, and estimated excretion rates of Indicator *E. coli* and *E. coli* O157. This is described below.

6.2.5 Livestock numbers in catchments

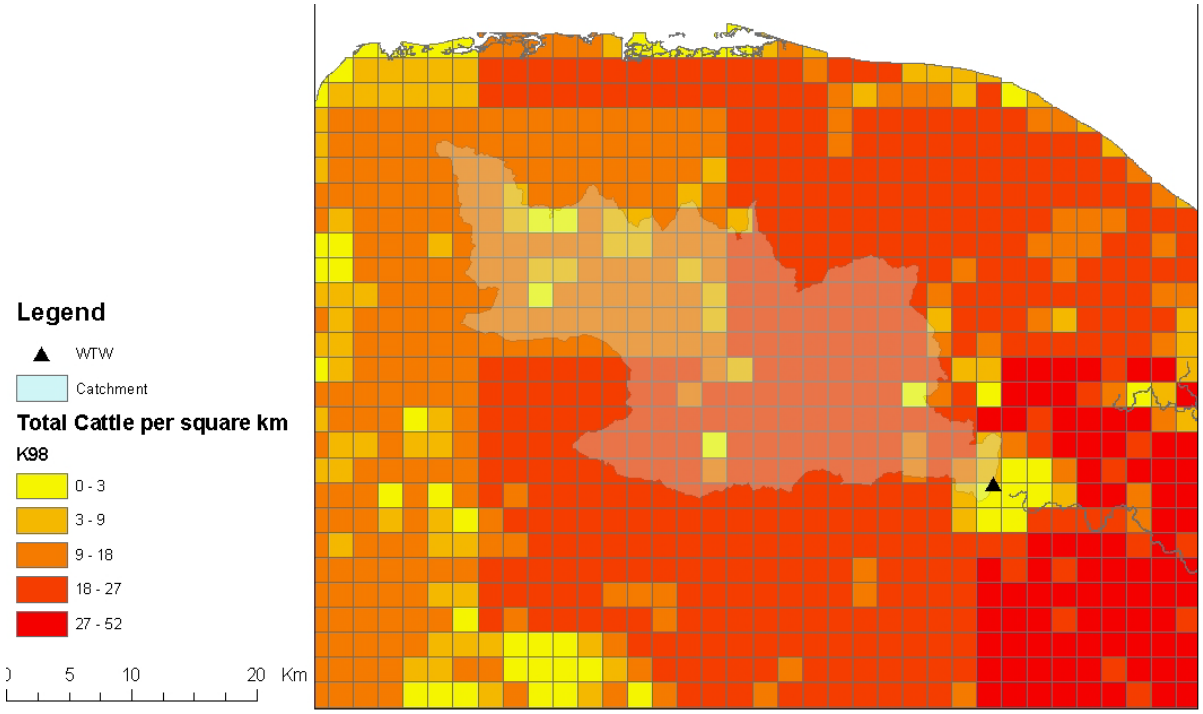
Estimation of catchment livestock numbers was performed in a number of stages. The first stage was to delineate the catchment of each surface water abstraction. A digital map of land heights (Hydrological Digital Terrain Model) was obtained from the NERC National Water Archive and these land heights used to calculate the catchment of each surface abstraction point using GIS. This process is illustrated in Figure 2 for surface water abstraction of the Heigham Water Treatment Works in Norwich (not one of the catchments used in this study). On this Figure the elevation is displayed in brown with lighter colours indicating higher elevations. The catchment is shaded in blue.

Figure 2: The Catchment of Heigham Water Treatment Works.



Once these catchments were delineated the number of livestock in each was estimated subdivided by animal type (sheep vs cattle) and age (age less than or greater than one year) to account for differences in manure volumes and shedding rates by animal type and age. The source of this is the DEFRA agricultural census supplied by the University of Edinburgh Data Library. This data source has combined agricultural census data together with land use data to estimate agricultural activities on a 2 km^2 cell. Using the GIS the total number of cattle (< and > 1 year) and the total number of sheep (< / > 1 year) were calculated for all the abstraction points and converted into densities per square km. This is illustrated in Figure 3 which displays the catchment for Heigham Water Treatment Works combined with information on cattle density.

Figure 3: Catchment for Heigham Water Treatment Works with Cattle Density



6.2.6 Excretion rates of *E. coli* O157 and indicator *E. coli* excretion from livestock

The final stage of the process was to convert these animal densities into *E. coli* 0157 densities. For each animal type (subdivided by age) the annual production of manure was estimated by multiplying with annual manure production figures for each. For cattle, manure production figures (faeces only) were obtained from Smith and Frost (Smith and Frost, 2000). Sheep manure production figures (faeces only) were obtained from Ogejo et al. (Ogejo et al., 2010). The concentration of *E. coli* 0157 in animal manure was then estimated using information on the percentage of animals likely to be positive for *E. coli* 0157 and the shedding intensity in *E Coli* 0157 within these manures. Because the available evidence indicates that the survival and transport of *E. coli* O157 in the environment is very similar to indicator *E. coli*, it can be assumed that the ratio of indicator *E. coli* : *E. coli* O157 is the same in freshly passed animal manure in the catchment as it is in water at the extraction point of the Water Treatment Works. This assumption is safe providing that livestock are the primary source of both indicator *E. coli* and *E. coli* O157, which is reasonable.

We have taken the concentration of indicator *E. coli* in manure from Ferguson et al. (Ferguson et al., 2007) and these are as follows:

Cattle: $10^{9.32}$ cfu *E. coli* / Kg

Sheep: $10^{10.4}$ cfu *E. coli* / Kg

We have followed the general approach of Dorner in modelling the excretion rates of *E. coli* O157 in two stages the first being the probability indicating whether or not the animal was colonised and the second a gamma distribution of counts in positive animals. We did not assume that the proportion

of livestock positive would be constant between different geographical regions but we did assume that the distribution of shedding intensities in positive animals would be the same.

To determine the proportion of livestock positive for *E. coli* O157 we extracted studies from the systematic review reported above. Unlike Dorner, however, we used a have used a meta-analytic approach to combine studies in a random effects model to generate a pooled estimate rather than a Bayesian approach. This was done using Comprehensive Meta-Analysis™. Only those cross sectional studies reported in table 4 were included in this analysis as other studies were restricted to Scotland. The pooled results are shown below (Table 14). For subsequent modelling we represented these probabilities as triangular distributions with the mean, lower and upper 95%iles.

Table 14: Pooled proportion of animals positive for *E. coli* O157 in studies in England and UK based on random effects models

Animal	No. studies	Proportion +ve	Lower 95%	Upper 95%
Cattle	4	0.064	0.036	0.114
Sheep	3	0.016	0.014	0.020

The distributions of intensities of excretion of *E. coli* O157 positive animals was taken from Dorner 2005 who represented the log distributions per g of fresh manure by a series of gamma distributions. These are listed below in table 15. For subsequent analyses we truncated the maximum limit of the gamma distributions to be the estimated total *E. coli* counts for the species given above.

Table 15: Parameters for Gamma distributions of intensity of shedding of *E. coli* O157 in positive samples (taken from Dorner)

Animal	Alpha	Beta
Calves	3.307	1.107
Adult cattle	1.853	1.492
Sheep	2.574	0.896

The Log of the ratio between indication and O157 is given by the equation

Log Ratio = Log *E. coli* O157 count – Log indicator count

The estimated log ratios for a positive animal determined by MonteCarlo modelling are shown as follows in table 16.

Table 16: Estimated log ratios for a positive animal determined by MonteCarlo modelling

Animal	Mean Log ratio	Std Deviation
Calves	-3.14	1.418
Adult cattle	-3.908	1.489
Sheep	-5.36	1.546

However, as effectively pointed out by Schijven and colleagues (Schijven et al., 2011) simply using the distribution from a single animal will over estimate variation in the mean of multiple samples. This has direct relevance to estimates of average excretion rates across herds of animals. Consequently the distribution log ratio for each livestock group was estimated as follows:

$$LR \sim Normal(\mu, \frac{s}{\sqrt{N}})$$

Where μ is the mean Log ratio and *s* the standard deviation of the single animal log ratio distribution from the above table. *N* is the estimated number of positive animals in the catchment. N was obtained by multiplying the actual number of animals in the catchment from table 17 by the probability of that animal group being positive.

The indicator *E. coli* excreted by an animal group was the product of the total manure production of that animal group in the catchment and the indicator *E. coli* excretion concentration from Ferguson et al. (2007) (see above). The distribution of *E. coli* O157 excretion per animal group was then given by the product of the total indicator *E. coli* and 10^{LR}. Both O157 and indicator *E. coli* excretion in a catchment was summed across the three livestock groups and the resulting ratio for each catchment then determined.

The indicator: *E. coli* 0157 ratio was then calculated. Table 17 shows the numbers livestock numbers and the log indicator :0157 ratios for each of the selected catchments and the private supplies.

Table 17: Livestock numbers for each of the catchment areas.

	Livestock numbers in catchment				Log O157:indicator *E. coli* ratios	
Code	**Cows**	**Calves**	**Sheep**	**Lambs**	**Mean**	**StdDev**
A1	1,562	480	108	64	-5.032	0.167
A2	886	338	190	147	-5.064	0.200
A3	906	354	432	469	-5.144	0.190
B1	0	0	2,819	2,445	-7.139	0.192
B2	0	0	0	0	-7.139[a]	0.192
B3	53,364	23,220	737,358	645,964	-6.425	0.041
B4	2,810	666	3,790	3,117	-5.380	0.124
C1	397	149	891	653	-5.380	0.122
C2	928	331	1,849	1,095	-5.519	0.231
C3	2,335	837	2,891	1,739	-5.317	0.129
D1	124,019	48,127	362,904	387,823	-5.660	0.071
D2	8,759	3,820	34,585	32,678	-5.064	0.199
D3	14,318	4,825	98,775	104,058	-6.106	0.058

[a] Given no livestock recorded in catchment ration taken from B1

For site B2, there was no recorded livestock in the catchment and consequently we used the ratio for site B1. For private water supplies we randomly sampled the O157:Indicator *E. coli* ratio from those of the 12 water utilities where a ratio have been calculated (i.e. excluding B2).

6.2.7 Estimating indicator *E. coli* concentrations in raw water

For all of the selected catchments we had data sets on indicator *E. coli* counts in raw water, and for private supplies we had data on indicator counts in water at the tap.

6.3 Private water sources

Rather than use the now historic PHLS dataset we were able to use the first year of data on private water supply monitoring provided by the drinking water inspectorate. This dataset had 5041 samples taken from 2672 sites in England and Wales. All the samples were taken during 2010. Of the 5041 sample results in the DWI private water supply dataset for 2010, *E. coli* were detected in 689 (13.7%). Indicating that 86.3% of samples passed the *E. coli* count requirements. This was substantially better than was the case in the late 1990s as found by Richardson et al. (Richardson et al., 2009). Of the positive samples the median count was 4 *E. coli*/100ml with the 10th percentile=1 and the 90th percentile=98. The distribution of *E. coli* counts is shown in figure 4.

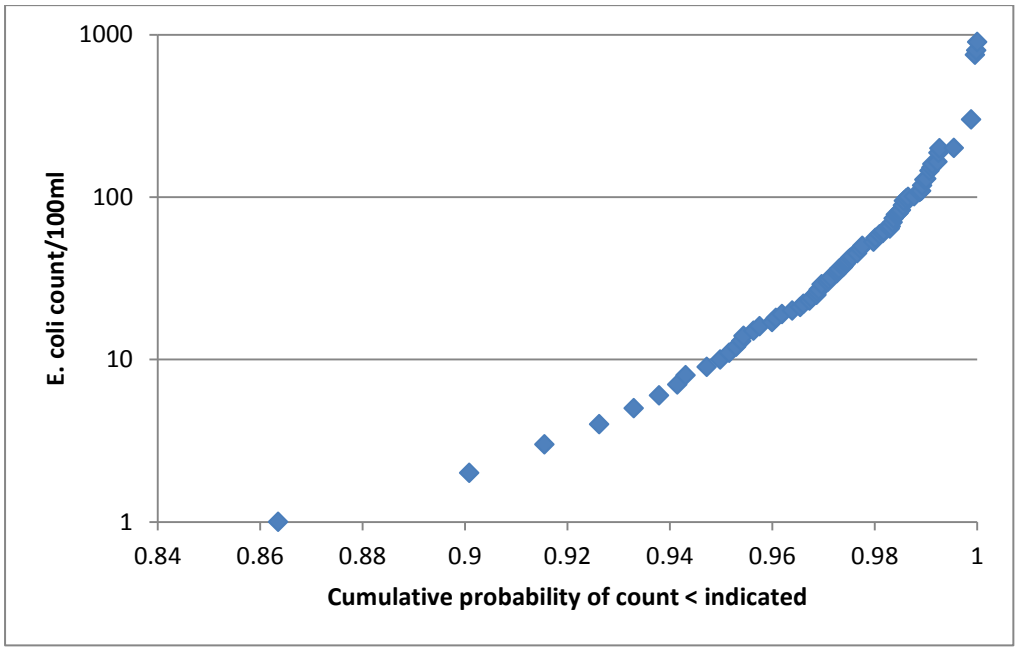
Figure 4: Distribution of *E. coli* counts from positive samples in the DWI private water supplies surveillance dataset 2010.

6.3.1 Sampling of indicator *E. coli* counts.

Indicator *E. coli* counts taken from the above data were randomly sampled for inclusion in the risk assessment. This was done by randomly sampling *n* in a uniform distribution between 0 and 1 and then taking the n^{th} percentile value. Where all, or most of a dataset contained positive results, ≥1 cfu/100ml this was sufficient. However, where many results were recorded as 0/100ml this could give an incorrect estimate of the concentration of indicator *E. coli*. Estimation of the actual concentration of indicator *E. coli* in raw water when the count was <1/100ml was done using a variation of the extrapolation method used by Hunter et al. (Hunter et al., 2011). However, in this context we examined a number of different models for predicting *E. coli* counts (not shown).

The best predictive model for predicting *E. coli* was done using a multi-level random effects model with Log10(*E. coli* count/100ml) as the outcome variable and the logit transformed cumulative probability of generating a count < the predicted Log10(*E. coli* count/100ml). This is best shown in figure 5.

Figure 5: Relationship between *E. coli* count/100ml and cumulative probability of having a count < given *E. coli* count (taken from private water supplies data).



So, for example, from the above graph 86% of samples had *E. coli* count <1/100ml, 90% of samples had count<2 and so on. Because these cumulative probability is a proportion we took the logit transformation for subsequent modelling.

Data from all sites and from private supplies were then incorporated into a multi-level random effects model with WTW site as the level variable, Log10(*E. coli*/100ml) as the dependant variable and logit transformed cumulative probability as the predictor variable . All private supply data was

treated as a single site. Note that the model was only constructed from observations where the *E. coli* count was >0/100ml. The best fit model results are presented below.

Box 1. Best fit model for prediction of *E. coli* counts<1 cfu/100ml

Performing EM optimization:
Performing gradient-based optimization:
Iteration 0: log likelihood = 2206.2957
Iteration 1: log likelihood = 2206.2957

Computing standard errors:

Mixed-effects ML regression	Number of obs = 3406
Group variable: wtw	Number of groups = 14
	Obs per group: min = 55
	avg = 243.3
	max = 688
	Wald chi2(1) = 166.08
Log likelihood = 2206.2957	Prob > chi2 = 0.0000

log10ecoli	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
newlogit	.4146686	.0321768	12.89	0.000	.3516032	.477734
_cons	1.320503	.2887122	4.57	0.000	.7546371	1.886368

Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
wtw: Independent				
var(newlogit)	.0144393	.0054755	.0068669	.0303619
var(_cons)	1.166739	.4412005	.5560235	2.448241
var(Residual)	.0150346	.0003658	.0143344	.015769

LR test vs. linear regression: chi2(2) = 12811.84 Prob > chi2 = 0.0000

Estimated best fit predictive equations for each of the sites taken from the random effects modelling is shown below, where the predicted *E. coli* count is given by:

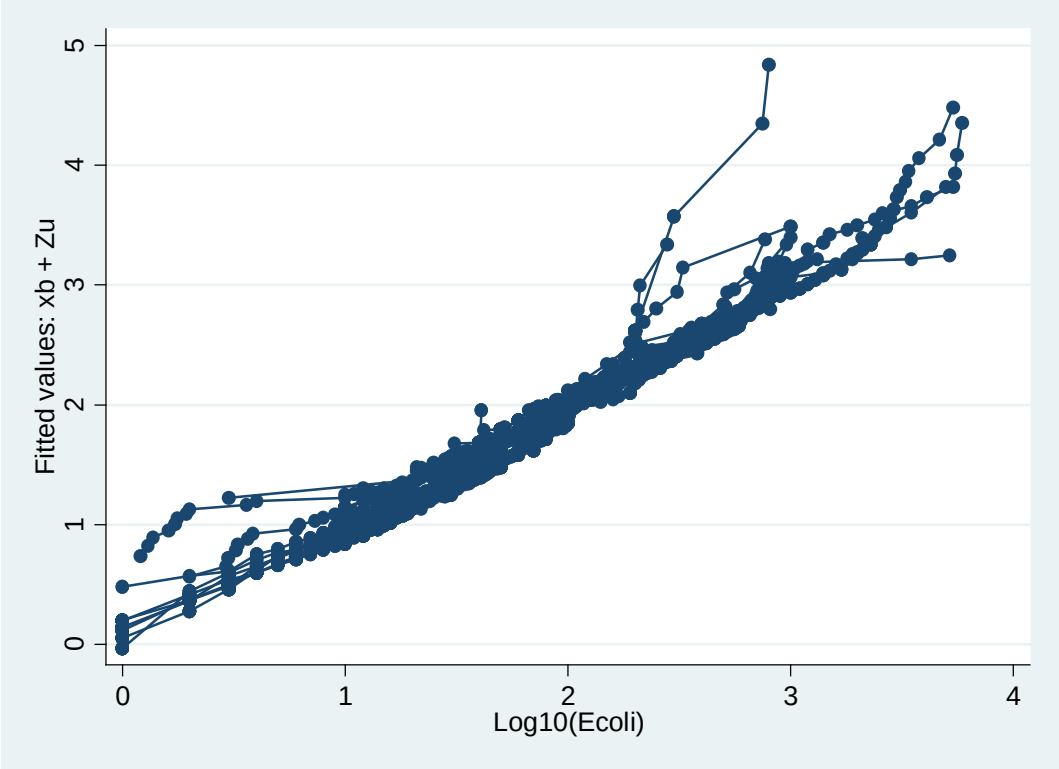
E. coli count for WTW)= a + b x (logit transformed cumulative probability).

Table 18: Random effects regression parameters for slope of indicator *E. coli* counts based on percentile

Water Treatment Works	Slope (b)	Intercept (a)
Private water supplies	0.708	-1.191
A1	0.297	2.520
A2	0.411	0.969
A3	0.393	1.653
B1	0.492	0.692
B2	0.539	-0.690
B3	0.384	2.192
B4	0.381	2.343
C1	0.341	1.956
C2	0.423	1.942
C3	0.448	1.747
D1	0.183	2.065
D2	0.495	0.632
D3	0.311	1.658

In order to test the validity of the above model we plotted predicted against actual counts where it can be seen that for the most part, the model gave very good predictions. The main areas of disagreement were at the extremes of the data for data sets with very few counts at the extreme.

Figure 6: Predicted *vs* actual indicator *E. coli* counts



Although the predictive value of this model is good within the range 1 to 10000 *E. coli*/100ml, it is still not possible to be certain of its applicability for counts below 1 *E. coli*/100ml. Indeed for many of the sites the model appears to over-predict counts at around the 1 *E. coli*/100ml level. Consequently we elected to use actual data for all sites and for private water supplies. For all data <1 *E. coli*/100ml we replaced the value with 0.1 *E. coli*/100ml. The data set was then randomly sampled for each *E. coli* value by randomly generating a number *N* between 0 and 1 and then taking the N^{th} percentile of the dataset.

The exception to this was for sites C1,2 and 3 as for most of the data set the limit of detection for *E. coli* counts were 10 and 1000/100ml. For these three sites we used the actual data as described above where they fell within the limits of detection and the above predictive model otherwise.

6.3.2 Water Treatment effectiveness

Estimates of water treatment effectiveness are taken from the final report of the MicroRisk project Smeets et al. (2006). Pre-chlorination efficiencies (Mean elimination capacity, MEC) taken from this report are shown in table 19. These efficiencies were modelled by triangular distributions with the median MEC and the range.

Table 19: Estimated mean elimination capacities of different treatment steps for indicator bacteria (Smeets et al. 2006).

	Studies	Data	Mean elimination capacity	50%ile	Range
Coagulation/floc removal	6	9	1.5	1.4	0.6 – 3.7
Rapid sand filtration	12	109	0.6	0.6	0.1 – 1.5
Granular activated charcoal	3	16	1.4	-	0.9 – 2.9
Slow sand filtration	9	17	2.7	2.4	1.2 – 4.8
Conventional treatment (coagulation-flocculation-sedimentation-filtration)	7	54	2.1	2.1	1 – 3.4
Direct filtration	4	35	1.4	1.5	0.8 – 3.3

Chlorination effectiveness was also taken from the MicroRisk report. The effectiveness of disinfection at different temperatures is given by the Arrhenius equation.

$$k_e = A \times e^{(\frac{-E_a}{RT})}$$

Where A is the frequency factor in l.mg-1.min-1, Ea is the activation energy (J.mol^{-1}), R is the ideal gas constant (8.314 J.mol^{-1}.K^{-1}) and T is the absolute temperature (K).

For this risk assessment, we used a K_e for *E. coli* of 6.67 $L.mg^{-1}.min^{-1}$ taken from the MicroRisk report which corresponds to that at 10°C. Also following the report we used the estimates based on a continuously stirred tank reactor (CSTR). The inactivation in a single CSTR is given by

$$\frac{N}{N_0} = \frac{1}{1 + k_e \times c \times t_h}$$

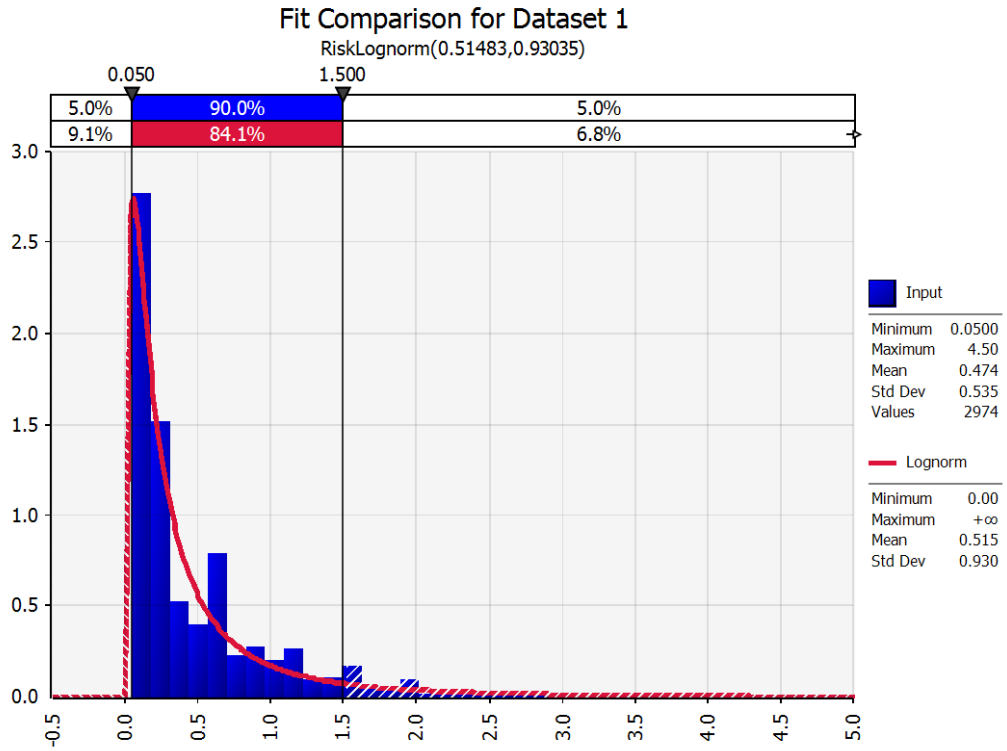
where N_o and N are the concentrations/L before and after the CSTR, c ($mg.L^{-1}$) is the disinfectant concentration at the outlet of the CSTR and t_h is the hydraulic residence time in the CSTR in minutes.

Given the range of chlorination policies reported for different utilities described above we modelled the chlorination effectiveness based on the least stringent policy of all the utilities questioned which equates to a CT of 15 $mg.L^{-1}$.min. This equates to a 2.005 log reduction in *E. coli* counts. This is very much a conservative estimate as even in the utility with this policy this is taken as an absolute minimum CT. For a CT of 30 $mg.L^{-1}$.min this would equate to a log reduction of 2.303.

6.3.3 Daily consumption of unboiled tap water

Daily drinking water consumption was taken from the recent reanalysis of the Addendum to the national tap water consumption report (Marsden, 2010). In this addendum, a table is given of the number of people consuming unboiled tap-water March/April and then again in June/July. Both these columns were combined and represented as a set of data-points with the mid-point value of the category from which they were derived. The distribution of daily water consumption is shown in figure 7 which also shows the optimal fitted curve, a log normal distribution with a mean of 0.515 and standard deviation of 0.930. For subsequent modelling sampled inputs were restricted to the maximum reported water consumption value contained in the DWI water consumption survey (4.5 L/day). When this truncation was applied the actual means and standard deviations for the sampled variable was 4.59 and 0.597 close to the actual distribution of 0.474 and 0.535

Figure 7: Distribution of daily consumption of unboiled tapwater and fitted model.



6.3.4 Dose –response curve

For the dose-response curve we used the Beta-Poisson model according to Teunis et al. (Teunis et al., 2004) and as recommended by the MicroRisk report. The parameters for this model were based on outbreak data with *E. coli* O157. Other proposed models for *E. coli* O157 were not based on *E. coli* O157 infection but *Shigella*. Teunis gave two models one for adults and one for children as follows:

	α	β
Adults	0.084	1.44
Children	0.050	1.001

Models for both children and adults were run.

6.3.4.1 Annual risk

The basic MonteCarlo model gives estimates of daily risk. To estimate annual risk we developed a further model with 365 input distributions corresponding to the daily risk. Risk was then summed over these 365 days to give the annual risk.

6.3.5 Risk with chlorination failure

To estimate the risk from a 24 hour failure in chlorination the models for each of the 13 WTW sites were re-run but with no account for chlorination. These gave daily risks with no chlorination. We then went onto estimate the impact that one days chlorination failure would have on annual risk. The annual risk was calculated as described above but with 364 normal and 1 chlorination failure days' risk models.

6.4 Results

We present the results of the Monte Carlo modelling of the risk of infection with O157 separately for private water supplies.

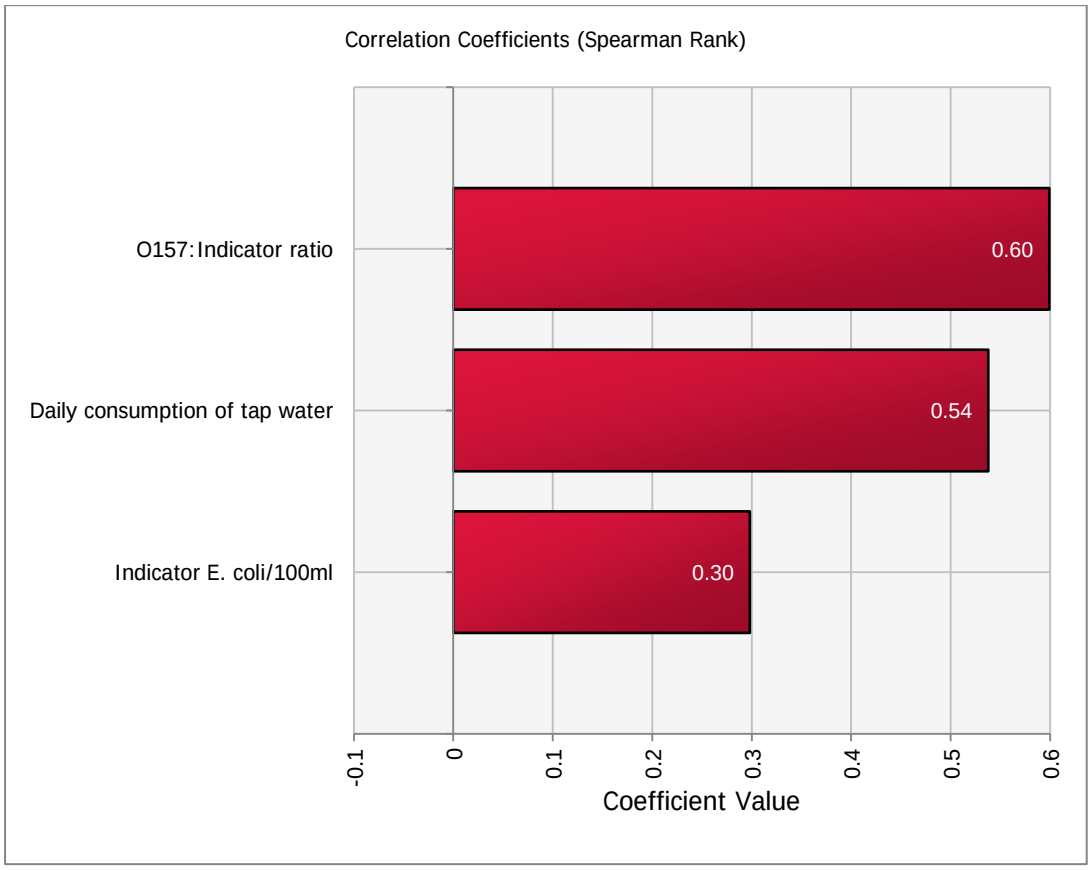
6.4.1 Private water supplies

For private supplies we present the results of for all samples and for data sets composed only of indicator negative and indicator positive *E. coli*. Table 20 gives the estimated *E. coli* O157 concentration in drinking water and daily intake of *E. coli* O157 through drinking water and daily and annual risks to consumers of water from private supplies. Given that daily risks in children and adults were very similar we have only calculated annual risks for adults which would marginally over-estimate risks for children. Figure 8 shows the Tornado plot for daily infection risk in Adults.

Table 20: Results of MonteCarlo modelling of Private water Supplies

Risk calculations based on	Result	Mean	Median	5%	95%
All samples	O157 conc/100ml	2.08E-05	4.57E-07	9.12E-09	3.08E-05
	Ecoli O157 per day	8.62E-05	1.05E-06	1.97E-08	9.08E-05
	Pinf/d / Adults	5.00E-06	6.11E-08	1.15E-09	5.30E-06
	Pinf/d / Children	4.27E-06	5.23E-08	9.82E-10	4.54E-06
	Annual risk in adults	4.72E-04	3.89E-04	2.30E-04	9.35E-04
Negative samples only	O157 conc/100ml	7.30E-07	2.49E-07	2.23E-08	2.79E-06
	Ecoli O157 per day	3.77E-06	6.27E-07	2.65E-08	1.39E-05
	Pinf/d / Adults	2.20E-07	3.66E-08	1.54E-09	8.11E-07
	Pinf/d / Children	1.88E-07	3.13E-08	1.32E-09	6.94E-07
	Annual risk in adults	8.14E-05	7.66E-05	5.52E-05	1.21E-04
Positive samples only	O157 conc/100ml	2.14E-04	1.36E-05	5.10E-07	8.01E-04
	Ecoli O157 per day	1.04E-03	3.63E-05	7.24E-07	3.08E-03
	Pinf/d / Adults	5.92E-05	2.12E-06	4.22E-08	1.80E-04
	Pinf/d / Children	5.03E-05	1.81E-06	3.62E-08	1.54E-04
	Annual risk in adults	2.19E-02	1.72E-02	9.71E-03	4.41E-02

Figure 8: Tornado graph showing impact of uncertainty and variation on estimated daily risk of STEC infection



It can be seen that the three input variables (O157:indicator ratio, daily unboiled tap water consumption and indicator *E. coli* concentration are all strongly associated with daily risk of infection. In none of these three input variables is variation driven primarily by uncertainty but actual geographic and temporal variation.

In conclusion, the mean annual risk in adults consuming unboiled tap water from private supplies is 4.72×10^{-4} or 5 cases per 10000 person years. However, almost all of this risk is experienced by people whose water quality fails the statutory *E. coli* standard. When the modelling was restricted to those supplies that complied with current standards the mean annual risk was estimated to be only 0.8 cases per 10000 person years. However, when the analysis was restricted to only those positive samples the risk was substantially greater equating to 219 cases per 10000 person years. Private water supplies in England do carry an important risk of STEC infection, although it would appear that this risk is driven largely by the minority of supplies that are not able to meet current legislative standards.

6.4.2 Public Water Utilities

The estimated concentrations of *E. c*oli O157 in drinking water, daily oral intake of *E. c*oli O157 and daily risk of symptomatic infection are shown in the following three tables (21-23).

Table 21: Estimated concentrations of *E. coli* O157/100ml in drinking water in each of the 13 Utility sites

Site code	Mean	Median	5%ile	95%ile
A1	5.57E-07	8.00E-08	2.32E-09	1.84E-06
A2	5.64E-07	7.35E-08	2.23E-09	1.81E-06
A3	1.17E-07	2.22E-08	8.65E-10	5.44E-07
B1	2.20E-08	2.99E-09	4.93E-11	9.35E-08
B2	2.03E-09	8.27E-11	3.60E-11	7.05E-09
B3	1.81E-08	4.17E-09	1.35E-10	7.56E-08
B4	3.00E-07	6.63E-08	1.76E-09	1.33E-06
C1	2.96E-07	6.59E-08	1.77E-09	1.29E-06
C2	1.36E-07	2.17E-08	1.23E-09	3.73E-07
C3	9.61E-07	2.51E-08	5.88E-10	1.06E-06
D1	3.09E-08	1.52E-08	1.49E-09	1.17E-07
D2	1.44E-07	2.63E-08	1.04E-09	6.64E-07
D3	8.59E-09	2.55E-09	1.04E-10	2.07E-08

Table 22: Estimated daily intake of *E. c*oli O157 from drinking water by consumers of water from each of the 13 Utility sites

Site code	Mean	Median	5%	95%
A1	2.96E-06	1.91E-07	3.54E-09	8.63E-06
A2	2.80E-06	1.80E-07	3.20E-09	8.21E-06
A3	5.40E-07	5.60E-08	1.23E-09	2.26E-06
B1	1.06E-07	7.16E-09	6.49E-11	4.40E-07
B2	1.06E-08	2.64E-10	2.53E-11	2.49E-08
B3	8.99E-08	1.01E-08	1.99E-10	3.50E-07
B4	1.64E-06	1.67E-07	2.52E-09	5.78E-06
C1	1.47E-06	1.60E-07	2.64E-09	5.63E-06
C2	6.83E-07	5.58E-08	1.76E-09	1.93E-06
C3	5.77E-06	6.40E-08	9.23E-10	4.21E-06
D1	1.65E-07	3.61E-08	1.79E-09	6.41E-07
D2	6.94E-07	6.61E-08	1.46E-09	2.86E-06
D3	4.56E-08	6.01E-09	1.33E-10	8.73E-08

Table 23: Estimated daily risk of symptomatic *E. c*oli O157 from drinking water by consumers of water from each of the 13 Utility sites

	Adults				Children			
Site code	Mean	Median	5%	95%	Mean	Median	5%	95%
A1	1.73E-07	1.11E-08	2.06E-10	5.04E-07	1.48E-07	9.54E-09	1.77E-10	4.31E-07
A2	1.63E-07	1.05E-08	1.87E-10	4.79E-07	1.40E-07	8.97E-09	1.60E-10	4.10E-07
A3	3.15E-08	3.26E-09	7.16E-11	1.32E-07	2.70E-08	2.80E-09	6.13E-11	1.13E-07
B1	6.19E-09	4.18E-10	3.79E-12	2.57E-08	5.30E-09	3.58E-10	3.24E-12	2.20E-08
B2	6.21E-10	1.54E-11	1.48E-12	1.45E-09	5.32E-10	1.32E-11	1.26E-12	1.25E-09
B3	5.24E-09	5.87E-10	1.16E-11	2.04E-08	4.49E-09	5.02E-10	9.93E-12	1.75E-08
B4	9.59E-08	9.73E-09	1.47E-10	3.37E-07	8.21E-08	8.33E-09	1.26E-10	2.89E-07
C1	8.57E-08	9.34E-09	1.54E-10	3.28E-07	7.34E-08	8.00E-09	1.32E-10	2.81E-07
C2	3.98E-08	3.25E-09	1.03E-10	1.12E-07	3.41E-08	2.79E-09	8.82E-11	9.63E-08
C3	3.35E-07	3.73E-09	5.38E-11	2.45E-07	2.87E-07	3.20E-09	4.61E-11	2.10E-07
D1	9.63E-09	2.10E-09	1.05E-10	3.74E-08	8.24E-09	1.80E-09	8.96E-11	3.20E-08
D2	4.05E-08	3.86E-09	8.52E-11	1.67E-07	3.47E-08	3.30E-09	7.29E-11	1.43E-07
D3	2.66E-09	3.51E-10	7.78E-12	5.09E-09	2.28E-09	3.00E-10	6.66E-12	4.36E-09

Figure 9: Tornado plot for WTW A1.

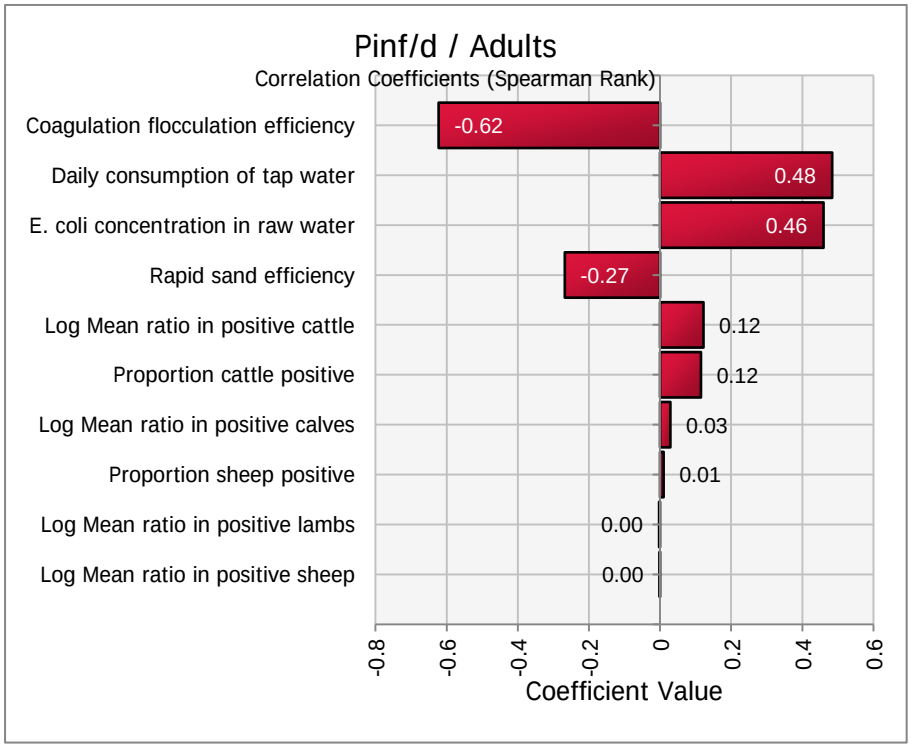
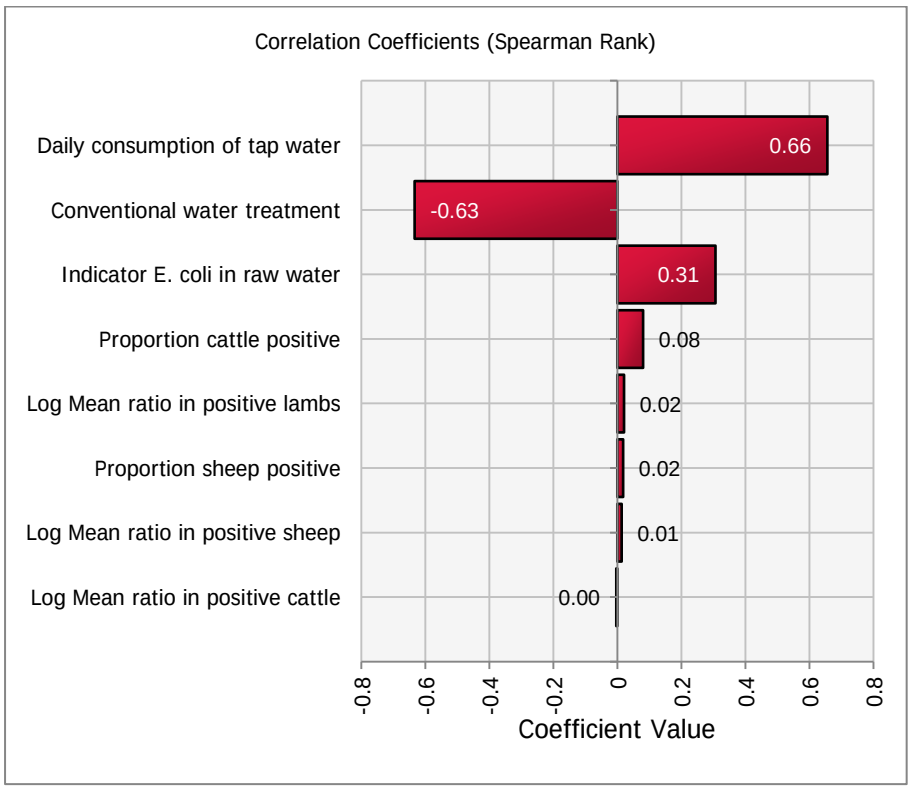


Figure 10: Tornado plot for WTW D1



It can be seen from both these plots (figures 9 and 10) that the main driver of variation in daily risk is daily drinking water consumption, variation in indicator *E. coli* concentrations in raw water and

variation/uncertainty in the removal efficiency of pre-chlorination water treatment. By contrast variation in the proportion of animals positive or shedding intensity has very little impact.

The calculated annual risk based on the mean daily risk for adults for the 13 water utilities is shown in table 24.

Table 24: Estimated annual risk of *E. coli* O157 infections from each of the drinking water case studies

Site code	Mean	Median	5%	95%	Estimated cases per 10,000 person years
A1	6.85E-05	5.75E-05	3.51E-05	1.30E-04	0.69
A2	6.55E-05	5.44E-05	3.27E-05	1.24E-04	0.66
A3	1.62E-05	1.41E-05	8.80E-06	2.94E-05	0.16
B1	5.51E-06	4.03E-06	2.08E-06	1.24E-05	0.055
B2	6.52E-08	5.97E-08	4.14E-08	1.03E-07	0.00065
B3	2.81E-06	2.44E-06	1.51E-06	5.05E-06	0.028
B4	5.10E-05	4.34E-05	2.65E-05	9.51E-05	0.51
C1	4.99E-05	4.26E-05	2.61E-05	9.34E-05	0.50
C2	1.18E-05	1.06E-05	7.09E-06	1.96E-05	0.12
C3	3.87E-05	2.97E-05	1.64E-05	7.95E-05	0.39
D1	4.03E-06	3.84E-06	2.82E-06	5.81E-06	0.040
D2	1.98E-05	1.70E-05	1.07E-05	3.61E-05	0.20
D3	1.37E-06	1.21E-06	7.84E-07	2.41E-06	0.014

The mean annual risk in the 13 water utility sites range from 6.52 $X10^{-8}$ to 6.85 $X10^{-5}$ or 0.00065 cases per 10000 person years in adults to 0.69 cases per 10000 person years. All water utilities are able to provide water with an annual risk of less than 1 per 10000 person years.

6.4.3 Estimating risk from chlorination failures

The above models were rerun for daily risk of illness for each of the sites but with the assumption of no effect of chlorination (i.e. a full 24 h failure in chlorination). Table 25 gives the estimated daily risk of illness during days when chlorination failed and also the impact on the estimated annual mean risk.

Table 25: Daily risk of infection in each of the Water Treatment works during a 24 hour chlorination failure and impact on annual risk

	Daily risk with no chlorination				Effect of 24 h chlorination failure on annual risk		Estimated cases per
Site code	Mean	Median	5%	95%	Mean	95%	10,000 person years
A1	1.51E-05	1.15E-06	2.07E-08	5.17E-05	8.66E-05	1.76E-04	0.87
A2	1.36E-05	1.10E-06	1.88E-08	4.35E-05	8.41E-05	1.68E-04	0.84
A3	3.72E-06	3.29E-07	7.12E-09	1.38E-05	2.07E-05	4.10E-05	0.21
B1	6.01E-07	4.34E-08	3.67E-10	2.58E-06	6.87E-06	1.55E-05	0.069
B2	5.42E-08	1.58E-09	1.50E-10	1.37E-07	8.20E-08	1.51E-07	0.00082
B3	6.07E-07	6.08E-08	1.04E-09	2.15E-06	3.65E-06	7.21E-06	0.037
B4	8.51E-06	9.87E-07	1.55E-08	3.43E-05	6.47E-05	1.31E-04	0.65
C1	9.19E-06	9.58E-07	1.63E-08	3.47E-05	6.51E-05	1.31E-04	0.65
C2	3.39E-06	3.17E-07	9.34E-09	1.17E-05	1.50E-05	2.76E-05	0.15
C3	1.64E-05	3.69E-07	5.42E-09	2.48E-05	4.91E-05	1.06E-04	0.49
D1	1.03E-06	2.15E-07	1.11E-08	3.81E-06	5.13E-06	8.85E-06	0.051
D2	4.17E-06	3.89E-07	8.05E-09	1.73E-05	2.56E-05	5.04E-05	0.26
D3	2.62E-07	3.51E-08	8.47E-10	9.96E-07	1.73E-06	3.33E-06	0.017

It can be seen that one day failure of chlorination does increase both daily and annual risk, though even in site A1 that has the highest risk the mean annual risk is still only 8.7 $X10^{-5}$ which corresponds to 0.87 cases per 10000 person years. Clearly if the failure in chlorination lasts for more than 24 hours then the estimated annual risk will soon exceed 1 case per 10,000 person years in several of the WTWs unless boil water notices are issued.

6.4.4 Test of model

The impact of two factors on the estimated risks has not so far been tested. The first is the choice of model parameters for the Beta Poisson distribution of the dose response curve and the second is the CT value. For all of the analyses to date we have used the parameters suggested by Teunis et al (2004). MicroRisk also suggested parameters by Powel et al (2000) where α=0.22 and β=8700. The second is the choice of a CT of 15 which as discussed above is conservative. Table 26 shows the impact of using the Powel parameters on the WTW with the midpoint daily risk. It can be seen that using using the Powell parameters for the Beta-Poisson model has a dramatic impact on the calculated risk (>3 log reduction). Whereas enhanced chlorination (a CT of 30) reduces daily risk by about 40%.

Table 26: **Impact of using Powell Beta-Poisson parameters and using enhanced chlorination on calculated daily risk of infection**

Risk assessment	Mean	Median	5%	95%
Base line (CT=15)	3.98E-08	3.25E-09	1.03E-10	1.12E-07
Using Powell parameters	1.74E-11	1.39E-12	4.15E-14	4.82E-11
With enhanced chlorination (CT=30)	2.39E-08	1.60E-09	4.83E-11	5.32E-08

7 Discussion

In this report we have undertaken a quantitative microbial risk assessment of the risk to human health from Shiga-toxin positive *E. coli* (STEC) also known as Vero-cytotoxigenic *E. coli* (STEC) or Enterhaemorrhagic *E. coli* (EHEC). Our results suggest that for public water systems the risk ranges between 0.00065 and 0.69 infections per 10,000 person years with a mean of about 0.26. For private supplies the risk is higher 4.7 infections per 10,000 person years. However, for private supplies complying with current *E. coli* standards the risk would be only 0.8 infections per 10,000 person years. This high risk associated with many private supplies is consistent with the findings of the survey of private water supplies reported above. In particular the high proportion of owners of such supplies without any adequate water treatment.

When undertaking any quantitative risk assessment there is usually an implicit setting of any results against some form of standard against which to judge the results as to whether or not the risk is acceptable/tolerable. There is no universally agreed standard by which any calculated risk can be said to be acceptable or not. What constitutes an acceptable level of risk is in many ways a political decision (Hunter, 2001). Consequently it is up to policy makers to make the decision about whether or not the results we have presented here would mean that current legislation provides adequate protection of public health or not. However, for this report we have generally taken the stance that a health risk should not exceed 1 infection per 10,000 consumers per year which is consistent with the UK's adoption of the World Health Organization's water safety approach. With the results above our conclusions are that English public drinking water supplies do not pose a significant risk of *E. coli* O157. However, overall private drinking water supplies do constitute an unacceptable risk except when they fully comply with the current indicator *E. coli* standards

In addition, the results of the analyses presented here must be compared to the known incidence of illness related to STEC O157 occurring in the British population. The recent second study of infectious intestinal disease in the community (IID2 Study) has provided the best estimates of such community illness rates to date (Tam, 2011). In the IID2 study the population incidence rate for STEC *E. coli* O157 was judged to be 3 cases per 10,000 person years but with fairly wide 95% confidence intervals (0 to 43). So taking the mean of the mean risks for the 13 WTWs (0.26 per 10,000 person years) our results would suggest that drinking water was responsible for just under 10% of all cases of STEC infections in the UK. Although plausible, this figure seems to be somewhat higher than what we would expect from the range of other risk factors described in existing case controlled studies on

sporadic infections, albeit ones conducted in the US (Denno et al., 2009, Voetsch et al., 2007). We will now turn our attention to discussing the validity of our results.

7.1 Validity

There are four key uncertainties in the risk analyses presented in this report.

1. One of the biggest problems with this risk assessment was obtaining valid exposure data. There is a dearth of studies that have attempted to estimate the concentration of *E. coli* O157 in raw or treated drinking water in England and Wales or indeed elsewhere. Even where studies have been reported in the literature data was rarely ever presented in a way that would be suitable for risk assessment. A significant problem with this study was, therefore, to develop estimates of *E. coli* O157 concentration in raw water or in private drinking water. Using the O157:indicator *E. coli* ratio in fresh manure as an indicator of the ratio in raw water was a reasonable approach given the available data suggesting that *E. coli* O157 has the same survival characteristics as indicator *E. coli*. However, for this study we only considered cattle and sheep. We elected not to include pig manure in the calculations because many pigs are kept indoors or in confined locations and so their manure should not enter the water course. Furthermore, as can be seen above probability of being positive and shedding intensity in positive pigs was lower than in cattle or sheep. So if we had included pig data then the estimated concentrations of *E. coli* O157 would be lower. It is likely that faecal contamination from other animals in water sheds would also have lower *E. coli* O157 excretion so our method for estimating *E. coli* O157 would over-estimate concentration.

2. It is likely that not all of the strains used in the Dorner thesis to generate the gamma distributions were likely to have been virulent. As can be seen in tables 3 to 5, not all *E. coli* O157 isolated from animals is likely to be virulent. Again we used the most conservative estimate which would over-estimate risk.

3. The chlorine contact times used in the analysis represented one the less stringent estimates and in any event the policy represents minimum chlorination intensity. It is likely that for most of the supplies studied actual chlorination would be rather greater. Once again our model is likely to over-estimate risk. Indeed doubling the CT (which represents policy for some utilities reduces daily risk estimates by about 40%).

4. The choice of the Teunis rather than the Powell Beta Poisson parameters has a major impact on estimated risk with the daily risk associated with the Powell model being 2 to 3 logs lower. Clearly we have taken the more conservative model again. However, as this model was based on data from human infections with STEC *E. coli* O157 rather than other pathogenic *E. coli* and *Shigella* species, we consider it to be more valid.

7.2 Ground water supplies

None of the WTWs modelled in this study were ground water treatment works. However, given the generally much higher microbiological quality of raw ground water than surface water conventional water treatment and chlorination would give substantially lower risks than for surface water. There is an issue for ground water supplies that received only chlorination. On the other hand in private supplies the risk associated with consumption of water meeting the *E. coli* standard was less than 1 case per 10,000 person years. Providing that raw water for groundwater supplies generally meets the *E. coli* standard then the risk should also be 1 case per 10,000 person years. The fact that even ground water supplies that remain *E. coli* negative are still chlorinated, the risk to public health will be substantially lower.

7.3 Contamination in distribution

We have not formally modelled risk to health from problems arising in distribution systems. In the MicroRisk study it proved very difficult to identify a risk to health through such contamination in distribution (van Lieverloo, 2006). When considering water quality at the tap as a whole in England and Wales, the proportion of tap water sample failing the *E. coli* standard is very small. During 2010 143,823 drinking water samples were collected at consumers taps and just 26 (0.018%) were *E. coli* positive (Chief Inspector of Drinking Water, 2011). Whilst is not possible to estimate risk of consumption of water at the tap we can be sure that contamination in the distribution network poses a risk of O157 infection that is substantially less than 1 case per 10,000 person years. However, any event in distribution that is followed by the detection of indicator *E. coli* at the tap could carry a risk of infection.

7.4 Other related STEC strains

This risk assessment only applies to STEC *E. coli* O157 strains. There are a wide range of other STEC positive strains that pose a risk to human health (Karch et al., 2005). There is much less information in the literature concerning the epidemiology and environmental distribution of non-O157 STEC in humans animals or water at least in the UK. In the English national surveillance system such non-O157 strains are much less frequently reported, though in the IID2 study they were detected in stool samples rather more frequently than O157 strains (Tam et al 2011). However, in IID2 no control samples were taken so it is not possible to determine what proportion of these strains were causing diarrhoeal disease. In the first IID study there were similar numbers of non-O157 STEC strains in cases and control samples suggesting that in majority of times these are isolated from stools they may not be clinically relevant (Food Standards Agency, 2000). However, numbers of positive

detections were very small. We consider there is insufficient data available on these Non-O157 strains to undertake a valid risk assessment

A particular concern has been the recent emergence in Germany of *E. coli* O104:H4 (HUSEC041) (Frank et al., 2011). This outbreak caused 4075 cases and 50 deaths across 15 countries. Many of the fatalities were in young adult women, probably reflecting this age/gender group's taste for raw sprouting seeds. This emergent pathogen does appear to be more virulent than other STECs. Most cases were traced back to a sprouting seeds factory in Germany. Contaminated drinking water was not implicated directly or indirectly in the outbreak. Nevertheless the possibility of future waterborne transmission of this virulent pathogen was discussed at a recent conference and a consensus statement released (Exner et al., 2011). It was noted that at the time of writing the new strain had only once been isolated from a surface water sample. It was also noted that there was no evidence of zoonotic transmission. Clearly if the strain remains absent from Europe then it will not pose a threat to water supplies. If the strain does return and becomes endemic in Europe, especially if it develops its reservoir in European Livestock, then it is certainly plausible that it would pose a threat to European drinking water supplies. However, given the assumption that it will respond to chlorine in the same way as indicator *E. coli* such risk would be small in properly managed WTWs. Like *E. coli* O157, any risk would fall most heavily on people consuming water from private supplies.

8 Conclusions

In conclusion, the risk to public health from STEC *E. coli* O157 from public WTWs in England varies from one supply to another depending largely on raw water quality and the proportion of cattle grazing in the water shed. However, for all WTWs studied the risk falls below 1 per 10,000 person years. For many supplies the risk falls well below this level, especially given the fact that the analyses presented here took a relatively conservative set of assumptions which is likely to over-estimate risk. Even when a 24 h failure in chlorination was modelled the risk fell below 1 per 10,000 person years in all WTWs. However, should there be several days of chlorination failure the annual risk could rise above this level for many of the WTWs. By contrast the risk associated with private supplies exceeded the acceptable risk level, though only for those supplies that that failed the *E. coli* standard.

9 Acknowledgements

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11 Appendices

11.1 Appendix 1: Medline (Ovid) search strategy

1. exp Water/
2. exp Water Microbiology/
3. exp Water Supply/
4. exp Fresh Water/
5. exp Water Pollution/
6. exp Water Purification/
7. exp Water Pollutants/
8. exp Mineral Waters/
9. river.ti,ab,kw.
10. rivers.ti,ab,kw.
11. lake.ti,ab,kw.
12. lakes.ti,ab,kw.
13. spring.ti,ab,kw.
14. springs.ti,ab,kw.
15. reservoir.ti,ab,kw.
16. reservoirs.ti,ab,kw.
17. wetland.ti,ab,kw.
18. wetlands.ti,ab,kw.
19. water.ti,ab,kw.
20. watercourse*.ti,ab,kw.
21. waterborne.ti,ab,kw.
22. watershed*.ti,ab,kw.
23. waters.ti,ab,kw.
24. waterworks.ti,ab,kw.
25. waterway*.ti,ab,kw.
26. freshwater*.ti,ab,kw.
27. greywater*.ti,ab,kw.
28. groundwater*.ti,ab,kw.
29. springwater*.ti,ab,kw.
30. surfacewater*.ti,ab,kw.
31. exp shiga-toxigenic escherichia coli/
32. exp Shiga Toxins/
33. Shiga*.ti,ab,kw.
34. enterohemorrhagic*.ti,ab,kw.
35. entero hemorrhagic*.ti,ab,kw.
36. enterohaemorrhagic*.ti,ab,kw.
37. entero haemorrhagic*.ti,ab,kw.
38. Verocytotox*.ti,ab,kw.
39. vero cytotox*.ti,ab,kw.
40. verotox*.ti,ab,kw.
41. vero tox*.ti,ab,kw.
42. "0157".ti,ab,kw.
43. 0157:H7.ti,ab,kw.
44. O157.ti,ab,kw.
45. O157:H7.ti,ab,kw.
46. VTEC.ti,ab,kw.
47. EHEC.ti,ab,kw.
48. STEC.ti,ab,kw.
49. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30
50. 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48
51. 49 and 50
52. exp cattle/
53. exp hemorrhagic syndrome, bovine/

54. cow.ti,ab,kw.
55. cows.ti,ab,kw.
56. cattle.ti,ab,kw.
57. livestock*.ti,ab,kw.
58. "dairy herd*".ti,ab,kw.
59. bovin*.ti,ab,kw.
60. heifer*.ti,ab,kw.
61. calf.ti,ab,kw.
62. calves.ti,ab,kw.
63. yearling*.ti,ab,kw.
64. steer.ti,ab,kw.
65. steers.ti,ab,kw.
66. bull.ti,ab,kw.
67. bulls.ti,ab,kw.
68. bullock.ti,ab,kw.
69. bullocks.ti,ab,kw.
70. ungulate*.ti,ab,kw.
71. 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70
72. exp sheep/
73. sheep.ti,ab,kw.
74. lamb*.ti,ab,kw.
75. ewe.ti,ab,kw.
76. ewes.ti,ab,kw.
77. ram.ti,ab,kw.
78. rams.ti,ab,kw.
79. caprin*.ti,ab,kw.
80. ovin*.ti,ab,kw.
81. ovis.ti,ab,kw.
82. 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81
83. exp swine/
84. pig.ti,ab,kw.
85. pigs.ti,ab,kw.
86. piglet*.ti,ab,kw.
87. gilt.ti,ab,kw.
88. gilts.ti,ab,kw.
89. sow.ti,ab,kw.
90. sows.ti,ab,kw.
91. boar.ti,ab,kw.
92. boars.ti,ab,kw.
93. porcine*.ti,ab,kw.
94. swine.ti,ab,kw.
95. 83 or 84 or 85 or 86 or 87 or 88 or 89 or 90 or 91 or 92 or 93 or 94
96. exp manure/
97. exp feces/
98. manure*.ti,ab,kw.
99. shedding.ti,ab,kw.
100. excreta.ti,ab,kw.
101. excrement*.ti,ab,kw.
102. droppings.ti,ab,kw.
103. feces.ti,ab,kw.
104. faeces.ti,ab,kw.
105. fecal*.ti,ab,kw.
106. faecal*.ti,ab,kw.
107. dung.ti,ab,kw.
108. waste.ti,ab,kw.
109. wastes.ti,ab,kw.
110. 96 or 97 or 98 or 99 or 100 or 101 or 102 or 103 or 104 or 105 or 106 or 107 or 108 or 109
111. (71 or 82 or 95) and 110 and 50
112. 111 not 51

11.2 Appendix 2: List of studies with full text reviewed and outcome of selection

Table S1: Livestock Included (5 publications, 4 unique studies):

ID Number	Reference	Duplicate studies
1090	(Chapman et al., 2001)	
1102	(Milnes et al., 2008)	
1095	(Milnes et al., 2009)	Duplicate study with Milnes et al., 2008
1096	(Paiba et al., 2002)	
819	(Paiba et al., 2003)	

Table S2: Livestock Excluded (53 publications):

ID Number	Reference	Reason for Exclusion
560	(Avery et al., 2004)	Naturally occurring indicator *E. coli*
524	(Berry and Wells, 2010)	Review
655	(Chase-Topping et al., 2007)	Study conducted in Scotland
644	(Chase-Topping et al., 2008)	Review
103	(Clough et al., 2003)	Modeling
236	(Duffy, 2010)	Review
1539	(Duffy et al., 2008)	Review
1494	(Duncan et al., 2000)	Review
1558	(Ellis-Iversen et al., 2007)	Farm-level prevalence reported only
19	(Ellis-Iversen and Watson, 2008)	Review
553	(Ellis-Iversen et al., 2008)	Farms initially selected on basis of being O157 positive
838	(Ellis-Iversen et al., 2009)	Farms initially selected on basis of being O157 positive
603	(Gunn et al., 2007)	Longitudinal study conducted in Scotland
753	(Halliday et al., 2006)	Duplicate
968	(Hutchison et al., 2004)	Waste samples (incl. bedding material and urine)
915	(Hutchison et al., 2005)	Duplicate
194	(Kerr et al., 2001)	Chosen on basis of link to human disease
501	(La Ragione et al., 2009)	Review
1220	(Lenahan et al., 2007)	Ireland
1311	(Liebana et al., 2005)	Longitudinal study
1446	(McEvoy et al., 2004)	Review
1229	(McGechan and Vinten, 2004)	Modeling
571	(Matthews et al., 2009)	Modeling
580	(Matthews et al., 2006a)	Modeling
645	(Matthews et al., 2006b)	Modeling

134	(Money et al., 2010)	Review
917	(Nicholson et al., 2004)	Review
605	(Ogden et al., 2004)	Study conducted in Scotland
1054	(Ogden et al., 2005)	Study conducted in Scotland
224	(Omisakin et al., 2003)	Study conducted in Scotland
339	(Pearce et al., 2004a)	Farm-level prevalence reported only. Study aim: distribution of O157 in bovine faeces.
647	(Pearce et al., 2004b)	Longitudinal study conducted in Scotland
646	(Pearce et al., 2009)	Duplicate
902	(Pedersen and Clark, 2007)	Review
1450	(Rhoades et al., 2009)	Review
599	(Robinson et al., 2004a)	Sampled animals known positive
1239	(Robinson et al., 2005)	Sampled animals known positive
660	(Robinson et al., 2009)	Modeling
304	(Robinson et al., 2004b)	Sampled animals known positive
638	(Shaw et al., 2004)	Duplicate study with Pearce et al., 2004b
226	(Smith et al., 2002)	Review
610	(Smith et al., 2010)	Sampled farms known positive
1083	(Solecki et al., 2009)	Study conducted in Scotland
1044	(Stacey et al., 2007)	Modeling
1520	(Stevens et al., 2002)	Review
1505	(Strachan et al., 2002)	Modeling
1435	(Strachan et al., 2001)	Outbreak
549	(Synge et al., 2003)	Sampled animals known positive
352	(Ternent et al., 2001)	Conference abstract only
1536	(Toft et al., 2005)	Modeling paper
198	(Vali et al., 2005)	Longitudinal study conducted in Scotland
1577	(Wood et al., 2007)	Modeling paper
776	(Zhang et al., 2010)	Modeling paper

Table S3: Water Included (31 publications, 29 unique studies):

ID Number	Reference	Duplicate studies
W280	(Ahmed et al., 2009)	
W493	(Astrom et al., 2007)	Duplicate with Deschesne and Soyeux 2007
W670	(Auckenthaler et al., 2002)	
W141	(Bonetta et al., 2010)	
W677	(Cooley et al., 2007)	
W32	(Dechesne and Soyeux, 2007)	
W480	(Diez et al., 2009)	
W315	(Dorner, 2005)	
W625	(Dorner et al., 2007)	Duplicate with Dorner 2005
W459	(Duris et al., 2009)	
W426	(Fincher et al., 2009)	

W382	(Fremaux et al., 2009)	
W44	(Gannon et al., 2004)	
W84	(Haack et al., 2009)	
W467	(Halabi et al., 2008)	
W410	(Heijnen and Medema, 2006)	
W285	(Heuvelink et al., 2008)	
W287	(Himathongkham et al., 2007)	
W281	(Jenkins et al., 2009)	
B148	(Johnson et al., 2003)	
W574	(Jokinen et al., 2010b)	
B144	(Jokinen et al., 2010a)	
W700	(Manandhar et al., 1997)	
W442	(Petterson et al., 2009)	
W384	(Savichtcheva et al., 2007)	
W214	(Schets et al., 2005)	
W289	(Shelton et al., 2006)	
B171	(Shelton et al., 2008)	
W104	(Smith et al., 2009)	
B117	(Urdahl et al., 2008)	
W283	(Wilkes et al., 2009)	

Table S4: Water Excluded (35 publications)

ID Number	Reference	Reason for Exclusion
W493	(Astrom et al., 2007)	Likely duplicate with Deschesne and Soyeux, 2007. No's slightly different.
W110	(Ahmad et al., 2009)	Review
B98	(Baker and Herson, 1999)	Review
W140	(Coffey et al., 2010)	Modeling (simulated)
W195	(Dharmasiri et al., 2010)	Insufficient data - Development of *E. coli* O157 detection method, total number of samples collected not reported. One *E. coli* O157 result reported: 4cfu/100mL.
B198	(Donnison and Ross, 2009)	Other - Survival in soil
W709	(Dorner et al., 2006)	Modeling (simulated)
W30	(Dorner et al., 2004)	Duplicate study
W231	(Ferguson et al., 2003)	Review
W640	(Ferguson et al., 2009)	Review
W484	(Ferianc et al., 2002)	Insufficient data - number of samples not reported.
W77	(Garcia-Aljaro et al., 2005)	Excluded water source (wastewater)
W27	(Foulds et al., 2002)	Indicator *E. coli*
B99	(Hashsham et al., 2004)	Review
W473	(Higgins et al., 2005)	Duplicate study

	(Kay et al., 2007)	Insufficient data - Total no. of samples collected/analysed for *E. coli* O157 not reported.
W483	(Lauber et al., 2003)	Insufficient data - No. of samples not reported/looking at gene fragments to predict O157 presence/absence. Insufficient data.
W433	(LeJeune et al., 2001)	Excluded water source (cattle trough water)
B170	(Little et al., 2003)	Indicator bacteria
W628	(Loge et al., 2002)	Excluded water source (storm drains)
B164	(McGee et al., 2000)	Conference abstract
B235	(Muniesa et al., 2006)	Review
W31	(Oliver et al., 2005)	Review
W728	(Olszewski et al., 2008)	Conference abstract
W612	(Quiett, 2005)	Paper only reports average CFU per site. Master's thesis.
W466	(Soller et al., 2010)	Modeling (simulated)
B100	(Stedtfeld et al., 2006)	Review
W19	(Stehman, 2000)	Review
B101	(Tourlousse et al., 2008)	Review
W179	(Watterworth, 2003)	Other - Lab-based study. Presence / absence of genes in strains of *E. coli.* Survival of O157 in inoculated/lab well water.
W717	(Welsh, 2007)	Sensitivity of method of detection used insufficient (2000 CFU/100ml)
W733	(Wojcicka et al., 2007)	Other - Effect of chlorine on inactivation of O157
B185	(Vinten et al., 2009)	Modeling
W216	(Looper et al., 2006)	Excluded water source (livestock water tanks)
W441	(Nwachuku and Gerba, 2008)	Review

11.3 Appendix 3: Inactivation of E.coli by free (available) chlorine

Table S5: Studies reporting the inactivation of *E. coli* O157:H7 by free (available) chlorine (shown in chronological order)

Reference	Experimental conditions	Outcome	Assessment
(Kaneko, 1998)	A patient strain of *E. coli* O157:H7 and a non-pathogenic stain *E. coli* K12 were used in this study. To provide sufficient numbers of cells, cultures were grown in nutrient broth. However, the cells were washed to remove any substances that could interfere with disinfection. Cells were added to a solution of free (available) chlorine (1.0 mg L^{-1}) at pH 7.2 and 30 °C, and the number of surviving bacteria examined over 10 minutes.	At an initial number of 100 cells 100 mL^{-1}, neither strain was detected after 5 minutes exposure to 1.0 mg L^{-1} of free (available) chlorine (FAC). Further experiments, with a greater number of cells, allowed a Ct (99 % inactivation) of 0.032 – 0.035 mg.min L^{-1} and a Ct (99.99 %) of 0.067 – 0.071 to be determined for clear water. In the presence of turbidity, created by the addition of kaolin (5 mg L^{-1}), a higher Ct (99%) of 0.04 – 0.05 mg.min L^{-1} and a Ct (99.99%) of 0.08 to 0.09 mg.min L^{-1} were required for the same extent of inactivation.	This study demonstrated that *E. coli* O157:H7 has a similar susceptibility to chlorination as typical strains of *E. coli*. A higher Ct was required to achieve equivalent inactivation in the presence of an interfering substance. However, the extent of this difference was small in comparison to the Ct values typically applied in water treatment.
(Lisle et al., 1998)	*E. coli* O157:H7 strain 932 was obtained the U.S. Environmental Protection Agency. A suspension of cells was prepared from a late-log phase, overnight culture in a medium without a carbon source for a period of 29 days. At specified time intervals over this period, the resistance to chlorination was determined by exposure to sodium hypochlorite solution at a final	The starvation conditions used in this study promoted the development of a cell type that was resistant to sub-lethal injury induced by membrane-active detergents (e.g., deoxycholate). Correspondingly, an increase in resistance to chlorine injury was observed which reached its maximum after 5 days starvation, and remained relatively constant through day 29.	This study has provided evidence that *E. coli* O157:H7 can develop resistance to chlorine concentrations up to 0.5 mg L^{-1}. However, this condition could only be induced by exposure for a short duration to chlorine concentrations, well below conditions encountered during water treatment.

	concentration of 0.5 ppm (mg L^{-1}) (as free (available) chlorine).		
(Rice et al., 1999)	Culture collection strains of *E. coli* O157:H7 originally isolated from cattle in the United States, and a range of wild-type ordinary *E. coli* strains obtained from a local source of cattle manure, chosen as strains that might contaminate water supplies after surface run-off from pastures and fields. All bacterial cultures were grown for 18 to 20 hours at 35 °C in a nutrient-rich broth. Cells were concentrated by centrifugation, and washed three times in phosphate buffer before testing. Initial cell number ranged between 5.5 to 5.6 $\log_{10}$ cfu mL^{-1}. The mean chlorine concentrations over the two minute exposure period were 1.1 mg L^{-1} free (available) chlorine and 1.2 mg L^{-1} total chlorine, prepared in a chlorine demand-free chlorinated (CDF) buffer at pH 7.0 and maintained at 5 °C. Viable bacteria were recovered on mT7 agar incubated for 22 to 24 hours at 35 °C. This medium was chosen because of its ability to recover oxidant-stressed organisms	For both the *E. coli* O157:H7 and the wild-type strains, exposure to this concentration of free (available) chlorine for one minute reduced the number of viable cells by approximately four orders of magnitude. These results indicate that the *E. coli* O157:H7 strains used in this study were sensitive to chlorination and were similar in resistance to that of wild-type *E. coli* isolates.	The study was undertaken in accordance with the general principles for testing the efficacy of a disinfectant. Limited range of experimental conditions makes it difficult to extrapolate to other situations in practice. The effectiveness of chlorine decreases with lower temperatures, broadly corresponding to the rate of chemical reactions as governed by the Arrhenius Equation. Consequently, inactivation would proceed twice as rapidly for each 10 °C rise in water temperature. The hydrogen ion concentration (pH) influences chlorination, and inactivation is less effective at higher pH values. The pH used in this study represents more ideal conditions likely to be encountered during water treatment, and inactivation would require a marginally longer period of time to achieve the equivalent reduction in cell number at higher pH values.
(Zhao et al., 2001)	Six isolates of *E. coli* O157:H7 of human origin recovered during an outbreak at a water park and a type strain of *E. coli* (ATCC 11229) were used as test organisms. All strains were cultured separately at	A free (available) chlorine concentration of 0.25 mg L^{-1} inactivated more than 10^7 cfu mL^{-1} of *E. coli* O157:H7 within 30 seconds. A period of 60 seconds was required to	The study was conducted following the basic principles for testing the efficacy of free chlorination.

	37 °C on nutrient agar. They were transferred at least three times at 24-hour intervals before use. Cells were harvested from nutrient agar plates, washed twice with 0.1 M phosphate buffer at pH 7.4, and suspended in the same buffer to achieve numbers of around 10^8 cfu mL^{-1}. For the inactivation test, a volume (1 mL) of each *E. coli* suspension was added to separate volumes (199 mL) of continuously stirred solutions of free (available) chlorine of 0.25, 0.5, 1.0, and 2.0 ppm (1 ppm is equivalent to 1 mg L^{-1}) maintained at 22 to 23 °C. At time intervals of 0, 0.5, 1.0, and 2.0 minutes a volume (1 mL) was removed, a neutralising agent added, and the remaining viable cells enumerated on eosin methylene blue agar medium after incubation at 37 °C for 24 hours. The identity of at least one colony from each plate was confirmed as *E. coli* O157:H7 by biochemical and immunological methods.	produce the corresponding degree of inactivation for the type *E. coli* (ATCC 11229). One particular *E. coli* O157:H7 strain from a sporadic case not associated with the outbreak at the water park was found to be more tolerant of chlorination. However, one strain was comparatively more resistant to chlorine at 23 °C for 1 minute, with a 4-, 5.5-, 5.8-, and 5.8-log_{10} cfu mL^{-1} reduction at free (available) chlorine concentrations (mg L^{-1}) of 0.25, 0.5, 1.0, and 2.0, respectively.	The majority of isolates of *E. coli* O157:H7 and the *E. coli* control strain were highly susceptible to inactivation by free (available) chlorine. The pH was in the range used for chlorination during water treatment. However, the temperature was at the maximum typically encountered for a surface source of water, during the summer months.
(Ryu and Beuchat, 2005)	Different *E. coli* O157:H7 strains; 43895-EPS (an exopolysaccharide (EPS) overproducing mutant), ATCC 43895+ (a curli-producing mutant) and ATCC 43895 were suspended in phosphate-buffered at 10^8 to 10^9 cfu mL^{-1}. The cells were exposed to free chlorine at concentrations of 0, 10, 25, and 50 mg L^{-1}. After 1, 3, 5, and 10 minutes, 2 mL of the chlorinated cell suspension was withdrawn and neutralised before	Strain 43895-EPS was more resistant than the other two strains to chlorine, indicating that protection was afforded by extracellular carbohydrate complexes (ECC). A 5.2 log_{10} cfu mL^{-1} reduction in numbers was observed after 10 minutes treatment with 10 mg L^{-1}F(A)C for cells producing lower amounts of ECC, whereas cells with higher amounts of ECC were unaffected by the same treatment.	This study was not carried out to examine directly the effects of chlorine on *E. coli* O157:H7 in relation to water treatment. Temperature and pH of chlorination was not reported. The efficacy of chlorination was probably being reduced by its reaction

	plating on TSA and incubated for 48 h at 37°C before colonies were counted.	Populations of cells of strains ATCC 43895+ and ATCC 43895 grown at both temperatures were reduced to <0.3 log_{10} cfu mL^{-1} within 1 minute exposure to 10 µg mL^{-1} F(A)C.	with the extracellular carbohydrate complexes. However, this effect would only become significant where low concentrations of chlorine and short contact periods are applied, i.e. a low Ct.
(Zhao et al., 2006)	Five isolates of *E. coli* O157:H7 of human and animal origin were obtained from an undisclosed location. Isolates were cultured in nutrient broth and washed cells were suspended in 0.1 M phosphate-buffered saline (PBS) at pH 7.2. Chlorine solutions were freshly prepared in deionised water. A volume (1 mL) of the cell suspension was added to a volume (199 mL) of 5 mg L^{-1} free (available) chlorine to add around 10^8 cells to the reaction vessel. The numbers of surviving bacteria were enumerated at defined time intervals over a period of 30 minutes. The disinfectant residual was neutralised and cells cultured on a nutrient rich medium for 24 hours at 37 °C.	Free (available) chlorine at 5 mg L^{-1} in water, in the absence of any interfering substances, inactivated 10^6 to 10^7 cfu mL^{-1} of *E. coli* O157:H7 to undetectable levels (1.7-log_{10} cfu mL^{-1}) in less than one minute.	Whilst, this study was not aimed at drinking water treatment, it demonstrated that free (available) chlorine was a highly effective for inactivation of *E. coli* O157:H7.
(Wojcicka et al., 2007)	One *E. coli* O157:H7 strain (ATCC 35150) was obtained from a culture collection and seven strains isolated from dairy farms and a lake in Wisconsin. Cells were cultured in a nutrient-rich medium, washed three times and suspended in sterile, deionised water adjusted to pH 7.0.	The *E. coli* O157:H7 strains were very sensitive to inactivation by free (available) chlorine. For most strains, a CT of less than 0.30 mg L^{-1}.min was sufficient to inactivate 2-3 log_{10} numbers of bacteria. The type strain was marginally more resistant to chlorination than three strains	The extent of the differences in the inactivation of the strains is small in comparison to the chlorination as practiced at a water treatment works. Unusually, most of the environmental isolates were more sensitive to inactivation than the reference strain.

	Chlorination was performed in buffered, deionised water at pH 7.0 at an initial free (available) chlorine concentration between 0.4 – 0.5 mg L^{-1}. The initial numbers of cells were between 10^7 to 10^8 cfu mL^{-1}. The numbers of cells were enumerated at time intervals of 15 – 30 seconds by counting colonies formed on nutrient agar after incubation for 24 hours at 37 °C.	isolated from the farm environment. One strain, however, was more resistant compared to all the other strains.	

ATCC – American Type Culture Collection
Cfu – colony forming units
F(A)C – free (available) chlorine