



Quantitative microbial human exposure model for faecal indicator bacteria and risk assessment of pathogenic *Escherichia coli* in surface runoff following application of dairy cattle slurry and co-digestate to grassland

Rajat Nag^{a,*}, Stephen Nolan^{b,c}, Vincent O'Flaherty^b, Owen Fenton^c, Karl G. Richards^c, Bryan K. Markey^d, Paul Whyte^d, Declan Bolton^e, Enda Cummins^a

^a University College Dublin School of Biosystems and Food Engineering, Belfield, Dublin 4, Ireland

^b National University of Ireland Galway, School of Natural Sciences and Ryan Institute, University Road, Galway, Ireland

^c TEAGASC, Environment Research Centre, Johnstown Castle, County Wexford, Ireland

^d University College Dublin School of Veterinary Medicine, Belfield, Dublin 4, Ireland

^e TEAGASC, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

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ABSTRACT

Animal waste contains high numbers of microorganisms and therefore can present a potential biological threat to human health. During episodic rainfall events resulting in runoff, microorganisms in the waste and soil may migrate into surface runoff, contaminating surface water resources. A probabilistic human exposure (HE) model was created to determine exposure to faecal indicator bacteria (FIB): total coliforms (TC), *E. coli* and enterococci following application of bio-based fertiliser (dairy cattle slurry, digestate) to grassland; using a combination of experimental field results and literature-based data. This step was followed by a quantitative microbial risk assessment (QMRA) model for pathogenic *E. coli* based on a literature-based dose-response model. The results showed that the maximum daily HE (HE_{daily}) is associated with *E. coli* for unprocessed slurry (treatment T1) on day 1, the worst-case scenario where the simulated mean HE_{daily} was calculated as 2.84 CFU day⁻¹. The results indicate that the overall annual probability of risk (P_{annual}) of illness from *E. coli* is very low or low based on the WHO safe-limit of P_{annual} as 10^{-6} . In the worst-case scenario, a moderate risk was estimated with simulated mean P_{annual} as 1.0×10^{-5} . Unpasteurised digestate application showed low risk on day 1 and 2 (1.651×10^{-6} , 1.167×10^{-6} , respectively). Pasteurised digestate showed very low risk in all scenarios. These results support the restriction imposed on applying bio-based fertiliser if there is any rain forecast within 48 h from the application time. This study proposes a future extension of the probabilistic model to include time, intensity, discharge, and distance-dependant dilution factor. The information generated from this model can help policymakers ensure the safety of surface water sources through the quality monitoring of FIB levels in bio-based fertiliser.

1. Introduction

Surface waters and groundwaters are, in principle, renewable natural resources (The European Commission, 2000). Although the application of raw farmyard manure and slurry (FYM&S) to arable land and grassland provides both economic and environmental benefits, including a reduced reliance on chemical fertilisers (Sokolova et al., 2018), it may pose a risk of microbial contamination of water sources.

Nutrient, metal and microbial loss in surface runoff is associated with applying treated sludge and dairy cattle slurry application to grassland soil (Peyton et al., 2016). The Nitrates Directive (Council Directive 91/676/EEC) aimed to reduce water pollution caused or induced by nitrates from agricultural sources and prevent further pollution (The European Commission, 1991).

North Atlantic European grassland systems have a low nutrient use efficiency and high rainfall (Nolan et al., 2020). This grassland is

* Corresponding author.

E-mail addresses: raj.nag@ucd.ie (R. Nag), stephen@glasportbio.com (S. Nolan), vincent.oflaherty@nuigalway.ie (V. O'Flaherty), owen.fenton@teagasc.ie (O. Fenton), karl.richards@teagasc.ie (K.G. Richards), bryan.markey@ucd.ie (B.K. Markey), paul.whyte@ucd.ie (P. Whyte), declan.bolton@teagasc.ie (D. Bolton), enda.cummins@ucd.ie (E. Cummins).

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typically amended with unprocessed slurry. Likewise, unprocessed slurry can be mobilised during rainfall events, contributing to pathogen, nutrient and metal incidental losses (Nolan et al., 2020). In an agricultural catchment, there are many potential faecal contamination sources, and the present study looked at the role of the application of raw FYM&S and anaerobic digestate (with or without pasteurisation) on land. Anaerobic digestion (AD) is a biological process that inactivates many pathogens through the process (Nag et al., 2019). Nolan et al. (2020) investigated incidental edge-of-field runoff losses following rainfall events monitoring the treatment types, application timing, and microbial counts in the runoff. The use of slurry has been identified as valuable feedstock for co-digestion reducing impacts of livestock production on the environment (Nolan et al., 2020).

Current EC legislation (S.I. No. 378 of 2006) mandates that inorganic and organic fertilisers or soiled water shall not be applied to land in any of the following circumstances to avoid contamination of surface water resources; (i) if the land is waterlogged, (ii) if the land is flooded or likely to flood, (iii) if the land is snow-covered or frozen, (iv) if heavy rain is forecast within 48 h, or (v) if the ground slopes steeply and, considering factors such as proximity to waterways, soil condition, ground cover and rainfall, there is a significant risk of causing water pollution (The European Union, 2006). Failure to comply with this legislation may result in microbial contamination of streams and lakes through surface runoff. Such contamination could result in subsequent water born disease if drinking water becomes contaminated. Such likelihood is increased because many pathogens are stable in water. For example, enteric bacteria (Gerba, 2008). Because routine examination of enteric pathogens' presence in water is often tedious, difficult, costly, and time-consuming; indicator organisms are typically used to assess the effectiveness of water treatment processes (Gerba, 2008).

Indicator microorganisms have traditionally been used as surrogate pathogens, and they are classified into three categories: process indicators, faecal indicators, and index and model organisms (Ashbolt et al., 2001). Process indicators demonstrate the efficacy of a process; for example, total coliforms (TC), are used for chlorine disinfection. Faecal indicator bacteria (FIB), such as faecal coliforms and, more specifically, *E. coli*, are a subgroup of total coliforms and indicate the presence of faecal contamination. Generic *E. coli* has been proposed by multiple scientific papers and risk assessors as FIB for enteric pathogens such as *Salmonella* spp. (Allende et al., 2018). Faecal coliform numbers in stabilised biosolids can be high, up to 10^5 CFU g⁻¹ dry matter. The survival time of FIB in the soil is not well established (Clarke et al., 2017; Schwarz et al., 2014), and to further complicate the issues, there are certain strains of *E. coli* that are capable of becoming naturalised in low-temperature environments of temperate maritime soils, persisting up to 9 years in soil (Brennan et al., 2010a, 2010b, 2010a).

Coliforms are present in high concentrations in human and animal digestive tracts and are excreted in faeces. Their presence in soil and the environment is generally indicative of faecal contamination. Coliforms can also persist and even multiply in the environment (Brennan et al., 2010b, 2013b; Brennan et al., 2013b; Brennan et al., 2010b; Moynihan et al., 2013). The inactivation of enteric pathogens is commonly assessed by quantitation of total coliforms, *E. coli*, and enterococci (Arnone and Walling, 2007; Saunders et al., 2012). Enterococci are tolerant of a wide range of environmental conditions and are easy to culture (Arnone and Walling, 2007).

While most coliform bacteria do not cause disease; some strains of *E. coli*, particularly Shiga toxin-producing *E. coli* (STEC) O157:H7, can cause serious illness (Nag et al., 2020a, 2020b, 2020b). This enterohaemorrhagic *E. coli* O157:H7 is the most important STEC serotype in relation to public health; however, other serotypes have frequently been involved in sporadic cases and outbreaks (WHO, 2018). Diseases and illnesses contracted from water with high faecal coliform counts include typhoid fever, hepatitis, ear infections, gastroenteritis, and dysentery (Clarke et al., 2017).

According to Regulation (EC) No.1069/2009 and Regulation (EU)

No. 142/2011, the allowable FIB levels for AD digestate must be < 1000 CFU g⁻¹ of fresh matter to avoid microbial pollution in surface water runoff (Jiang et al., 2018; Nolan et al., 2018). However, Nolan et al. (2020) reported that unprocessed dairy cattle slurry, unpasteurised digestate, and even soil may exceed the allowable concentration of 1000 CFU g⁻¹ for some FIB. Therefore, it is important to conduct a risk assessment study to assess the potential impact of pathogen runoff to surface water sources under a range of circumstances following the application of animal waste on agricultural land.

During the AD process, complete inactivation of pathogens in the digestate is unlikely (Nag et al., 2019). Persistence may be related to factors such as temperature, pH, and the water content of treated sludge; in addition, there is often regrowth of pathogens post-treatment, known as the 'regrowth' or 'reactivation' phenomenon (Clarke et al., 2017). The explanations for reoccurrence may be linked to i) incomplete inactivation of organisms in the AD plant, ii) contamination from external sources during post-digestion, termed 'bypass' in continuous or semi-continuous stirred-tank reactors (Longhurst et al., 2013), iii) a large drop in temperature after AD or post-AD pasteurisation conditions (Clarke et al., 2017; Nag et al., 2019).

The efficacy assessment of drinking water treatment plants (DWTP) in eliminating potential pathogens resulting from agriculture-associated runoff is important. Efficacy of DWTP removal of microorganisms at various stages of treatment has been reported. Cummins et al. (2010) described a conventional water treatment shown in Fig. 1. Conventional treatment is known to reduce the numbers of *Cryptosporidium* oocysts and *Giardia* cysts by an average of 99.950% (3.17 log₁₀ reduction) and 99.993% (4.14 log₁₀ reduction), respectively (Rose et al., 1996).

1.1. Primary treatment (log₁₀ reduction in drinking water treatment plant (primary treatment) or LR_{DWTP,P})

Primary treatment consists of large debris (sticks, stones, rubbish and other large objects) screening and grit removal from a ground or surface water source (Cummins et al., 2010). It may also involve a period of storage, pre-conditioning, and pre-chlorination. As screening is only designed to remove large objects, the impact on pathogen removal is negligible (Clarke et al., 2017) and was not considered a significant pathogen removal process source.

1.2. Secondary treatment (LR_{DWTP,S})

Secondary treatment involves removing fine solids and most contaminants using filters, coagulation, flocculation and membranes (Cummins et al., 2010). As reported in Cummins et al. (2010), The chemicals/methods used in secondary DWTP in Ireland are alum coagulation (10 water treatment plants), FeCl₃ coagulation (1), flocculation (12), aeration (4), dissolved air flotation (2), clarification (3), sedimentation (24), slow sand filtration (8), rapid gravid filters (28), membrane filtration (4). The term 'no filtration or not indicated' was indicated for 65 plants. The majority of Irish water treatment plants implement a secondary treatment stage using coagulation/flocculation with alum, coupled with sedimentation.

1.3. Tertiary treatment (LR_{DWTP,T})

In Ireland, most of the public water supply involves the use of chlorine treatments (73 plants monitored). One plant uses ozone for the disinfection process (Cummins et al., 2010). A 2 to 3 log removal of faecal indicators such as *E. coli* and *Salmonella* is common (Gerba, 2008; Safe Drinking Water Committee and National Research Council, 1980), but this may be higher (Bailey et al., 2018) as the log reduction per 100 ml when dual disinfection treatments with chlorine is used may achieve a reduction in *E. coli*, *Enterococcus* spp., *Salmonella*, and *Clostridium perfringens* of 6.3, 5.5, 4.5, and 4.5, respectively.

According to the European Drinking Water Directive 98/83/EC,

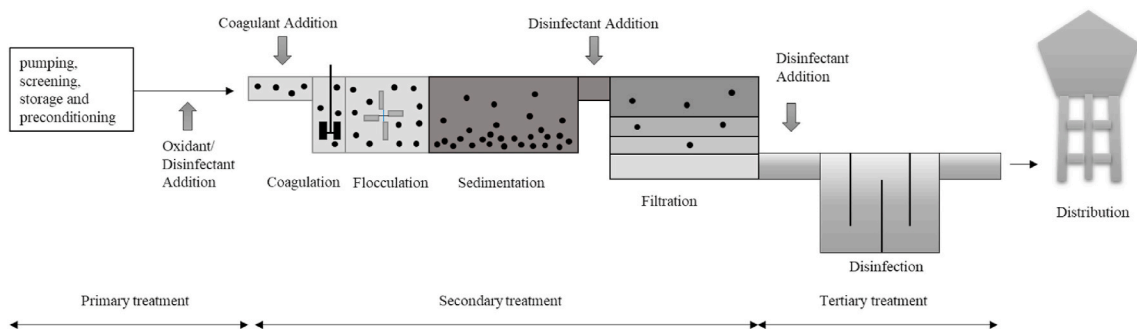


Fig. 1. The conventional water treatment process comprising of coagulation, flocculation, sedimentation, filtration, and disinfection.

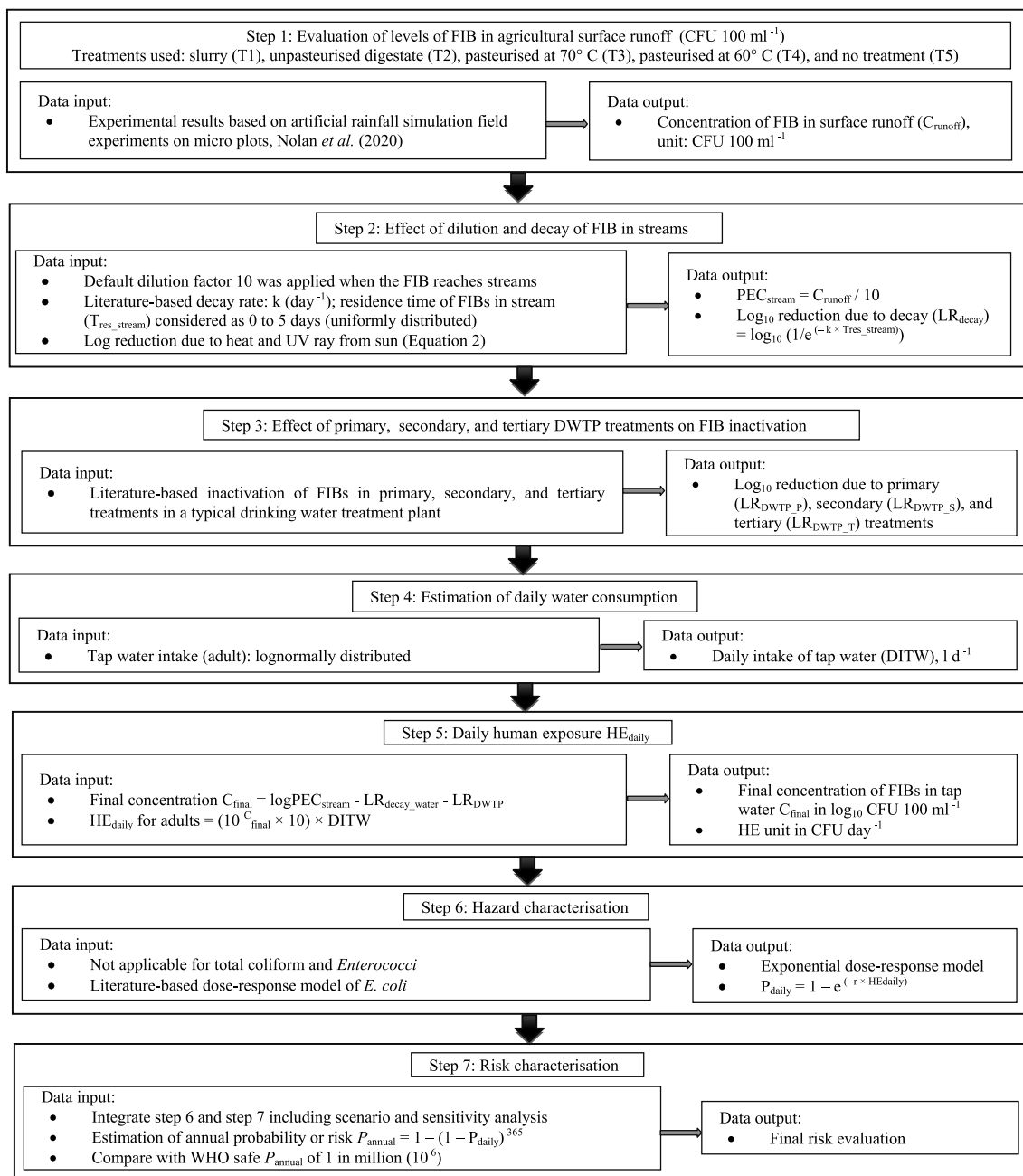


Fig. 2. A schematic framework for the QMRA model.

drinking water entering the distribution system should contain zero coliforms, *E. coli*, and enterococci in 100 ml⁻¹ (The European Commission, 1998). Therefore, it is critical to determine the FIB burden associated with the land application of FYM&S and anaerobic digestate (with or without pasteurisation), the potential for environmental diffusion and transport, and potential human exposure (HE). Also, *E. coli* enteropathogenic (EPEC) has been found to be among the primary pathogens of concern from anaerobic digestate on agricultural lands in Ireland (Nag et al., 2020a, 2020b, 2020b).

The overall objective of the present study was to develop a probabilistic HE/quantitative microbial risk assessment (QMRA) model for FIBs/pathogenic *E. coli* in surface water to consider the consequence of the environmental fate of FIBs following dilution during storm events, the residence time in surface water, die-off in water, the likely effect of drinking water treatment (DWT), and human consumption of drinking water.

2. Materials and methods

2.1. Model structure and development

A QMRA model was created to analyse the HE to target FIBs from drinking water. Fig. 2 shows the main steps involved in developing the model for this study and highlights the data input/output associated with each step. Step 1 involves evaluating FIB loading in surface runoff from the agricultural field treated with different bio-based fertiliser treatments. The detailed methodology of the rainfall simulation experiment is documented in Nolan et al. (2020) and the supplementary material (Fig. A1). Four treatments were examined in this study, along with a control (no treatment) scenario. These treatments were dairy cattle slurry (DCS); and three types of AD digestate, namely unpasteurised digestate; digestate with EU pasteurised guidelines (1 h at 70 °C); and digestate with Irish pasteurised condition (96 h at 60 °C). The co-digestion was based on dairy cattle slurry: waste from a food processing facility (Fat Oil And Grease - FOG) as 2 : 1 (Nolan et al., 2020). A comparison was carried out to see the influence of different types of animal waste (dairy cattle slurry vs unpasteurised/pasteurised digestate) on the microbial count in the runoff. All digestates were sourced from the same semi-continuously fed, mesophilic, continuously stirred tank bioreactors running at 37 °C. DCS was collected from a dairy farm in Co. Galway, Ireland, following mechanical agitation of the underground slurry tank. Fresh DCS and digestates were collected in sealed, 10 l-capacity plastic storage containers and transported to the field site, where they were briefly stored at 4 °C before application. Four treatments of dairy cattle waste were applied to fields: slurry (T1), unpasteurised digestate (T2), post-AD pasteurisation at 70 °C for 1 h (T3), post-AD pasteurisation at 60 °C for 96 h (T4), and a control field received no treatment (T5). The next step (2) looked at the dilution and decay of microorganisms in surface water based on a default dilution factor and first-order die-off rates. Next, scientific literature-based log reduction during different stages of DWTP was collated in step 3, while step 4 considered a probabilistic intake of tap water by adults. The HE_{daily} was calculated in step 5 and was compared using literature-based dose-response models (hazard characterisation) in step 6. In the final step (7), risk characterisation also looked at several scenarios and sensitivity analysis enabling risks to be prioritised by assigning risk estimates to each link (node) in the exposure chain and identifying appropriate risk management strategies.

2.2. Estimation of FIB concentration in surface runoff (C_{runoff})

Initial FIB levels in the surface water runoff were based upon micro-plot runoff experiments conducted by Nolan et al. (2020). The initial concentrations of FIB in surface water (C_{runoff}) from micro plots after different application treatments such as dairy cattle slurry (T1), unpasteurised anaerobic digestate (T2), digestate with post-AD

pasteurisation at 70 °C (T3), digestate with post-AD pasteurisation at 60 °C (T4), and untreated control (T5) were used. A lognormal distribution was fitted to the mean and standard deviation of the experimental results (Table 1).

2.3. Effect of dilution in streams (LR_{decay_water})

A dilution factor (DF) is the ratio of the concentration of pollutant in the effluent to its concentration in the receiving water after mixing in the receiving water (USGS, 2011). According to the European Commission (2003), a default dilution factor (DDF) of 10 may be applied if no specific data are available. The dilution factor is a function of flow rates which can be a function of precipitation and the total discharge flow from the catchment into streams. Due to the different seasonal, climatic, and geographical conditions in the Member States, DDF may vary widely, i.e. from 1 (e.g. dry riverbeds in summer) up to 100,000 (The European Commission, 2003). A DDF of 10 can be used as a pessimistic approach to avoid such uncertainties, as reflected in studies (Clarke et al., 2017; Keller et al., 2014; Kim et al., 2010), who reported the dilution factor of 10 for similar climatic condition. Therefore, a DDF of 10 was applied to the C_{runoff} to calculate the predicted environmental concentrations (PEC_{stream}) in stream water (Eq. (1)) as also reported in Clarke et al. (2017), the unit of PEC_{stream} is CFU 100 ml⁻¹.

$$PEC_{stream} = \frac{C_{runoff}}{DDF} \quad (1)$$

2.4. Effect of decay of FIBs in streams (LR_{decay_water})

Decay in water is another aspect of the model. As reported in Clarke et al. (2017), a 5-day residence time in streams is sufficient to inactivate microorganisms exposed to sunlight. Therefore, a uniform distribution, 0–5 days, was adopted (Clarke et al., 2017) to model the variability of time of travel or residence time of FIBs in streams (T_{res_stream}). The decay (first-order reaction rate constant) values of FIBs in streams were adopted from literature as presented in Table 2. Also, Lewerin et al. (2018) indicated that decay is the sum of microbial die-off and growth, depending on temperature and nutrient availability. The final FIB count (after decay) can be calculated by Eq. (2).

$$C_t = C_0 \times e^{-k t} \quad (2)$$

C_t is the concentration at time t , C_0 the initial concentration and k (unit: day⁻¹) the first-order inactivation rate constant or daily die-off value. Log reduction or LR ($\log_{10}(C_0/C_t)$) can be derived from Eq. (2), substituting t equals to T_{res_stream} (day), LR_{decay} can be equated from Eq. (3).

$$LR_{decay} = \log_{10} \frac{C_0}{C_t} = \log_{10} \left(\frac{1}{e^{-k \times T_{res_stream}}} \right) \quad (3)$$

2.5. Effect of FIB reduction in DWTP (LR_{DWTP}): secondary ($LR_{DWTP_S_co/\text{fl}}$) and $LR_{DWTP_S_fil}$) and tertiary (LR_{DWTP_T}) treatments

A cumulative sum of FIB removal (LR_{DWTP}) was calculated based on log reduction at individual steps involved in a DWTP. An appropriate distribution was adopted for LR_{DWTP} and is presented in Table 4, which was further used for the QMRA.

$$LR_{DWTP} = LR_{DWTP_S_co/\text{fl}} + LR_{DWTP_S_fil} + LR_{DWTP_T} \quad (4)$$

where.

$LR_{DWTP_S_co/\text{fl}}$ is the log reduction due to coagulation/flocculation in a secondary treatment plant.

$LR_{DWTP_S_fil}$ stands for the log reduction due to filtration in the secondary treatment plant, and.

LR_{DWTP_T} means the log reduction due to disinfection with free chlorine (mostly) in a tertiary treatment plant.

Table 1

Fitted distributions on the concentration of FIBs in surface water (C_{runoff}) from micro plots applied with different treatments*; Lognormal (mean, standard deviation) unit CFU 100 ml⁻¹.

Time (days post-application prior to rain)	The concentration of FIBs (CFU 100 ml ⁻¹) in surface water (C_{runoff}) from different treatments applied				
	T1 Slurry	T2 Unpasteurised digestate	T3 Post-AD pasteurisation at 70 °C for 1 h	T4 Post-AD pasteurisation at 60 °C for 96 h	T5 Control
TC					
1	Lognormal (6.42 × 10 ⁶ , 5.41 × 10 ⁵)	Lognormal (1.02 × 10 ⁵ , 5.96 × 10 ⁴)	Lognormal (3.52 × 10 ⁴ , 1.70 × 10 ⁴)	Lognormal (5.07 × 10 ⁴ , 3.43 × 10 ⁴)	Lognormal (2.14 × 10 ⁴ , 1.68 × 10 ⁴)
2	Lognormal (1.08 × 10 ⁵ , 1.12 × 10 ⁵)	Lognormal (3.54 × 10 ⁴ , 5.91 × 10 ⁴)	Lognormal (2.45 × 10 ³ , 2.65 × 10 ³)	Lognormal (1.06 × 10 ⁴ , 1.85 × 10 ⁴)	Lognormal (1.53 × 10 ⁴ , 1.53 × 10 ⁴)
14	Lognormal (1.42 × 10 ⁴ , 9.68 × 10 ³)	Lognormal (2.98 × 10 ³ , 4.44 × 10 ³)	Lognormal (1.60 × 10 ³ , 1.66 × 10 ³)	Lognormal (7.94 × 10 ² , 4.91 × 10 ²)	Lognormal (8.36 × 10 ² , 6.61 × 10 ²)
30	Lognormal (1.50 × 10 ³ , 1.15 × 10 ³)	Lognormal (4.83 × 10 ² , 2.80 × 10 ²)	Lognormal (9.00 × 10 ² , 9.06 × 10 ²)	Lognormal (3.09 × 10 ² , 2.86 × 10 ²)	Lognormal (3.45 × 10 ² , 3.14 × 10 ²)
E. coli					
1	Lognormal (3.26 × 10 ⁵ , 2.08 × 10 ⁵)	Lognormal (5.00 × 10 ⁴ , 7.67 × 10 ⁴)	Lognormal (7.87 × 10 ³ , 7.05 × 10 ³)	Lognormal (6.40 × 10 ³ , 9.13 × 10 ³)	Lognormal (3.12 × 10 ³ , 3.34 × 10 ³)
2	Lognormal (7.09 × 10 ⁴ , 9.53 × 10 ⁴)	Lognormal (3.76 × 10 ⁴ , 7.47 × 10 ⁴)	Lognormal (1.25 × 10 ³ , 5.18 × 10 ²)	Lognormal (7.69 × 10 ² , 6.49 × 10 ²)	Lognormal (4.46 × 10 ² , 7.62 × 10 ²)
14	Lognormal (5.33 × 10 ³ , 4.14 × 10 ³)	Lognormal (2.01 × 10 ³ , 3.68 × 10 ³)	Lognormal (6.67 × 10 ² , 1.24 × 10 ³)	Lognormal (8.00 × 10 ¹ , 9.10 × 10 ¹)	Lognormal (1.00 × 10 ⁰ , 3.00 × 10 ⁰) ^a
30	Lognormal (4.65 × 10 ² , 6.15 × 10 ²)	Lognormal (6.70 × 10 ¹ , 1.24 × 10 ²)	Lognormal (1.34 × 10 ² , 2.34 × 10 ²)	Lognormal (8.20 × 10 ¹ , 9.20 × 10 ¹)	Lognormal (7.00 × 10 ¹ , 1.37 × 10 ²)
Enterococci					
1	Lognormal (1.67 × 10 ⁵ , 2.91 × 10 ⁵)	Lognormal (9.24 × 10 ² , 1.45 × 10 ³)	Lognormal (3.46 × 10 ² , 2.21 × 10 ²)	Lognormal (8.48 × 10 ² , 1.01 × 10 ³)	Lognormal (1.88 × 10 ² , 8.70 × 10 ¹)
2	Lognormal (1.81 × 10 ⁴ , 1.91 × 10 ⁴)	Lognormal (1.26 × 10 ⁴ , 2.48 × 10 ⁴)	Lognormal (4.95 × 10 ³ , 9.82 × 10 ³)	Lognormal (1.25 × 10 ⁴ , 2.47 × 10 ⁴)	Lognormal (2.52 × 10 ³ , 4.98 × 10 ³)
14	Lognormal (5.56 × 10 ³ , 8.77 × 10 ³)	Lognormal (1.21 × 10 ² , 6.80 × 10 ³)	Lognormal (1.31 × 10 ² , 7.80 × 10 ¹)	Lognormal (2.50 × 10 ² , 2.10 × 10 ²)	Lognormal (3.78 × 10 ² , 4.32 × 10 ²)
30	Lognormal (8.21 × 10 ³ , 1.41 × 10 ⁴)	Lognormal (3.10 × 10 ¹ , 3.00 × 10 ¹)	Lognormal (6.10 × 10 ¹ , 4.20 × 10 ¹)	Lognormal (1.56 × 10 ² , 1.16 × 10 ²)	Lognormal (2.15 × 10 ² , 1.84 × 10 ²)

Note.

*Detail methodology of field experiments can be found in Nolan et al. (2020) and Peyton et al. (2016).

^a) The lower limit of detection for FIBs was 1 CFU 100 ml⁻¹ and the upper limit of detection was 10⁷ CFU 100 ml⁻¹.

Table 2

Decay* or die-off constant of different FIBs in the aquatic environment.

FIBs	Experiment temperature (°C)	Observation period (days)	Parameter value/method (day - ¹)	The equation used or log reduction LR _{decay}	Reference
TC	Mean 12 (8–23)	5	k = 0.0444 to 0.2773	Eq. (2)	Perkins et al. (2016)
<i>E. coli</i>	Mean 12 (8–23)	5	k = 0.0048 to 0.0627	Eq. (2)	Perkins et al. (2016)
<i>Enterococcus</i> spp.	Mean 12 (8–23)	5	k = 0.0052 to 0.3163	Eq. (2)	Perkins et al. (2016)

Note: *Decay rates of faecal indicator bacteria are based on sewage and ovine faeces in brackish and freshwater in North Wales, UK (Perkins et al., 2016).

Table 3

Calculated log reduction for FIBs in three stages of DWTP.

FIBs	Coagulation/flocculation (LR _{DWTP,S_{co/f}})/ sedimentation/activated sludge	Filtration (LR _{DWTP,S_{fil}})/ membrane filtration	Tertiary (LR _{DWTP,T})/ disinfection with combined and free chlorine	Reference	Distribution considered for model LR _{DWTP} = (LR _{DWTP,S_{co/f}}) + (LR _{DWTP,S_{fil}}) + (LR _{DWTP,T})
TC	2.2–2.4	1.6–4.4	0.7–3.5	Scott et al. (2003)	Uniform (4.5, 10.3)
<i>Enterococcus</i> spp.	1.8–2.9	1.2–3.2	0.7–2.9	Scott et al. (2003)	Uniform (3.7, 9)
<i>E. coli</i>	4.97 (Membrane bioreactor + activated sludge followed + sand filtration + UASB)		2–3	(Gerba, 2008), (Safe Drinking Water Committee and National Research Council, 1980)	Uniform (6.97, 7.97)

2.6. Estimation of daily intake of tap water (DITW)

A lognormal distribution for daily water consumption in Ireland for the adult population was adopted with a mean and standard deviation value of 0.564 ± 0.617 l d⁻¹ (5th percentile, mean and 95th percentile values as 0.088, 0.563, and 1.637 l d⁻¹, respectively) based on a survey

conducted by the Irish Universities Nutrition Alliance (IUNA, 2011). It was assumed that the population drinks solely tap water as the added complexity of allowing for bottled water consumption and the use of filtration or UV systems in domestic homes was beyond this paper's scope.

Table 4
WHO limit of risk (WHO, 2001)..

Number	Population	Risk classification
1	in 1,000	Very high
1	in 10,000	High
1	in 100,000	Moderate
1	in 1,000,000	Low
1	in > 1,000,000	Very low

2.7. Human exposure HE (daily)

The concentration of FIBs, C_{final} (unit, \log_{10} CFU 100 ml⁻¹), in drinking tap water was calculated by Eq. (5). All the terms of Eq. (5) have been explained in sections 2.2 to 2.5. The HE_{daily} (CFU day⁻¹) was calculated by multiplying the number of FIB in 1 l drinking water by DITW (Eq. (6)). Exposure assessment was conducted for 3 FIBs (TC, *E. coli*, and enterococci) looking at the surface water transportation pathway. Hazard characterisation was only possible for *E. coli* as the dose-response models are not applicable for the other two FIBs as they are typically non-pathogenic.

$$C_{final} = \log_{10} PEC_{stream} - LR_{decay_{water}} - LR_{DWTTP} \quad (5)$$

$$HE_{daily} = (10^{C_{final}} \times 10) \times DITW \quad (6)$$

2.8. Hazard characterisation and dose-response models to evaluate P_{daily} and P_{annual}

Hazard characterisation looked at the probabilistic daily response (P_{daily}) due to HE based on literature-based dose-response models, where dose means HE_{daily} and response stands for illness and r represents the probability of a single cell of the pathogen causing infection or illness through the oral route (Eq. (7)). It should be noted from DuPont et al. (1971), cited in (CAMRA, 2019), that the matrix of oral ingestion r equals 9.7×10^{-9} for *E. coli* (including pathogenic and non-pathogenic (Nag et al., 2021)).

$$P_{daily} = 1 - e^{(-r \times HE_{daily})} \quad (7)$$

This P is the daily probability (P_{daily}) which was used to calculate the annual probability (P_{annual}) (Eq. (8)). A Monte Carlo simulation method was run (10,000 iterations) using @RISK 7.6 software (PALISADE corporation) which is an add-in to Microsoft Excel 2016 to get the minimum (5th percentile), mean, and maximum (95th percentile) values of P_{annual} (illness).

$$P_{annual} = 1 - (1 - P_{daily})^{365} \quad (8)$$

2.9. Scenario analysis and classification of risk

A scenario analysis was performed to evaluate the influence of different treatments. A total of 60 (20 × 3) scenarios, including 20 (4 × 5) for each FIB, were performed at four-time intervals (1 d, 2 d, 14 d, 30 d) for 5 treatments. Next, 5-tier risk classification categories for P_{annual} (Eq. (8)), namely: very high, high, moderate, low, and very low, were adopted based on WHO (2001) and documented in Table 4. Furthermore, it must be noted that the response highlighted in WHO (2001) is used regularly with regards to deaths due to chemical pollutants. The limit of 1 in 1 million (1 in 10^6) is widely used as a safe limit of low or 'no risk'.

3. Results and discussion

3.1. HE_{daily}

The estimated HE_{daily} to TC, *E. coli*, and enterococci are displayed in Table 5. The highest HE_{daily} was observed for *E. coli* for treatment T1 on day 1 followed by TC for treatment T1 on day 1, *E. coli* for T1 on day 2, for T2 on day 1, and T2 on day 2. In only one of 60 (20 × 3) cases, the simulated mean HE_{daily} exceeded 1 CFU day⁻¹, and that was for *E. coli* (T1 & d 1).

3.2. Annual probability of risk (P_{annual})

The overall P_{annual} of *E. coli* was assessed as very low or low. One scenario, the raw slurry application on day 1, resulted in a moderate risk, which is the worst-case scenario (Table 6). Unpasteurised digestate (T2) showed low risk on days 1 and 2; however, there is very low risk due to surface runoff after day 2. Pasteurised digestate (T3) indicates very low P_{annual} while in only one scenario, the 95th percentile touched the low-risk bar. Beyond day 2, there is very low risk associated with *E. coli* in surface runoff irrespective of all treatments, which supports the restriction imposed on the application of bio-based fertiliser to avoid spread if there is any forecast of rain in the following 48 h from the time of application.

3.3. Infectious dose comparison between overall and pathogenic *E. coli*

Relative proportions of bacteria comprising the faecal coliform group are not always the same; therefore, dose-response models are not available for the bacterial groups' cumulative effect. For example, TC is a group of Gram-negative bacteria found in soil, contaminated surface water and faeces comprising four genera of the *Enterobacteriaceae* family; *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella* (Fewtrell and Bartram, 2001). Faecal coliforms (mainly *E. coli*) are a subgroup of TC and are found in the gut and faeces of people and animals (Clarke et al., 2017). Gastrointestinal pathogens such as enteropathogenic and enterohaemorrhagic *E. coli* (EPEC and EHEC) threaten human health worldwide (Hartland and Leong, 2013). The infectious dose (ID_{50}) in a 50% healthy population was calculated as 5.6×10^3 for enterohaemorrhagic *E. coli* serotype O157 compared to 7.1×10^7 for *E. coli* serotypes overall (Fig. 3). Therefore, the illness model could not be parameterized to assume that the severity of the enteric pathogen *E. coli* O157 is similar to *E. coli* (overall), which could be an overestimation. Conversely, the overall *E. coli* count ratio and pathogenic *E. coli* count have been calculated as 1 : 0.08 (Daley et al., 2019). If we apply the same ratio to the HE_{daily} and use the same exponential dose-response model, the simulated mean P_{annual} of pathogenic *E. coli* for treatment T1 on day 1 would be 7.7×10^{-7} (5th percentile 3.9×10^{-11} , 95th percentile 4.0×10^{-6}) which is one 13th of the simulated mean P_{annual} of the baseline T1-day 1 scenario of *E. coli*. In all other scenarios, the P_{annual} was very low risk (even 95th percentile) due to pathogenic *E. coli*.

3.4. Sensitivity analysis

A sensitivity analysis was performed around the HE_{daily} output to look at the most influential parameter due to input uncertainty and variability. From Fig. 4, it was evident that log removal at drinking water treatment plant is the most sensitive parameter for TC and *E. coli*, and it is negatively correlated with the HE_{daily} having Spearman's rank-order correlation coefficient - 0.96 for both. In contrast, C_{runoff} is the most sensitive parameter for enterococci which is positively correlated (coeff. + 0.7). Daily intake of tap water was the second most sensitive parameter (positively correlated, + 0.19 for TC and +0.23 for *E. coli*) followed by C_{runoff} (+0.18 for TC and +0.15 for *E. coli*). Tap water consumption was the second most sensitive parameter for enterococci (coeff. + 0.53) followed by LR_{DWTTP} (- 0.4). The die-off constant k and

Table 5
Daily human exposure (HE_{daily}) to TC, *E. coli*, and enterococci.

Treatment	Time (days post-application prior to rain)	HE _{daily}								
		TC			<i>E. coli</i>			Enterococci		
		5th percentile	Mean	95th percentile	5th percentile	Mean	95th percentile	5th percentile	Mean	95th percentile
T1	1	0.00	0.58	2.84	0.00	2.84	13.88	0.00	0.00	0.01
T2	1	0.00	0.09	0.45	0.00	0.46	1.90	0.00	0.00	0.00
T3	1	0.00	0.03	0.15	0.00	0.06	0.31	0.00	0.00	0.00
T4	1	0.00	0.04	0.23	0.00	0.05	0.23	0.00	0.00	0.00
T5	1	0.00	0.01	0.09	0.00	0.02	0.12	0.00	0.00	0.00
T1	2	0.00	0.09	0.41	0.00	0.62	2.66	0.00	0.00	0.00
T2	2	0.00	0.03	0.12	0.00	0.33	1.17	0.00	0.00	0.00
T3	2	0.00	0.00	0.01	0.00	0.01	0.05	0.00	0.00	0.00
T4	2	0.00	0.01	0.03	0.00	0.00	0.03	0.00	0.00	0.00
T5	2	0.00	0.01	0.06	0.00	0.00	0.01	0.00	0.00	0.00
T1	14	0.00	0.01	0.05	0.00	0.04	0.22	0.00	0.00	0.00
T2	14	0.00	0.00	0.01	0.00	0.01	0.07	0.00	0.00	0.00
T3	14	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
T4	14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T5	14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T1	30	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
T2	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T4	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T5	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: The values of more than 0.1 CFU are highlighted in bold.

Table 6
Probabilistic estimated P_{annual} due to *E. coli* burden in drinking water..

Treatment	Time (days post-application prior to rain)	5th percentile	Mean	95th percentile	Risk (5th percentile)	Mean risk	Risk (95th percentile)
T1	1	4.721E-10	1.008E-05	4.914E-05	Very low	Moderate	Moderate
T2	1	3.854E-11	1.651E-06	6.752E-06	Very low	Low	Low
T3	1	9.401E-12	2.266E-07	1.101E-06	Very low	Very low	Low
T4	1	5.146E-12	1.91E-07	8.201E-07	Very low	Very low	Very low
T5	1	3.242E-12	9.083E-08	4.333E-07	Very low	Very low	Very low
T1	2	5.815E-11	2.203E-06	9.427E-06	Very low	Low	Low
T2	2	1.933E-11	1.167E-06	4.162E-06	Very low	Low	Low
T3	2	1.986E-12	3.786E-08	1.848E-07	Very low	Very low	Very low
T4	2	9.32E-13	2.416E-08	1.136E-07	Very low	Very low	Very low
T5	2	0	1.349E-08	5.432E-08	Very low	Very low	Very low
T1	14	7.213E-12	1.667E-07	7.929E-07	Very low	Very low	Very low
T2	14	1.094E-12	6.091E-08	2.512E-07	Very low	Very low	Very low
T3	14	0	1.986E-08	7.868E-08	Very low	Very low	Very low
T4	14	0	2.2E-09	1.093E-08	Very low	Very low	Very low
T5	14	0	3.791E-11	1.473E-10	Very low	Very low	Very low
T1	30	0	1.323E-08	6.198E-08	Very low	Very low	Very low
T2	30	0	1.975E-09	7.707E-09	Very low	Very low	Very low
T3	30	0	4.118E-09	1.543E-08	Very low	Very low	Very low
T4	30	0	2.629E-09	1.131E-08	Very low	Very low	Very low
T5	30	0	2.077E-09	7.687E-09	Very low	Very low	Very low

T_{res,stream} displayed a very small value of correlation coefficient (negatively correlated), which signifies that these parameters' variability does not play a significant role compared to the other three inputs. Enterococci are not easily inactivated by heat treatment (Nolan et al., 2018), so the influence of LR_{DWTP} was less for enterococci suggesting that they are a better indicator for spore-forming heat resistant bacteria. For example,

Watcharasukarn et al. (2009) highlighted that *Clostridium perfringens* is the most heat-resistant organism followed by *Enterococcus faecalis*, while *E. coli* is the most heat-sensitive organism. Therefore, non-pathogenic FIB enterococci may be a reasonable indicator for *Clostridium*.

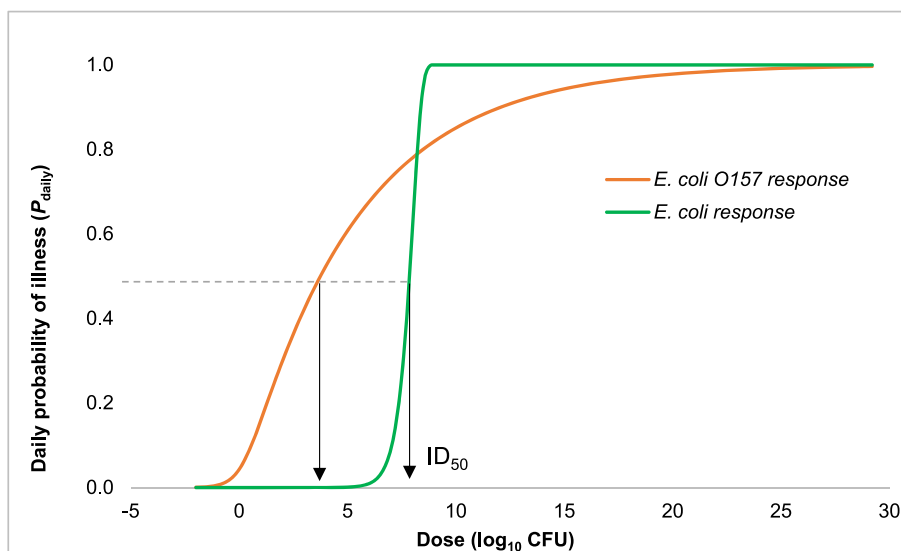


Fig. 3. Exponential model for *E. coli* (DuPont et al., 1971), parameter $r = 9.7 \times 10^{-9}$; and Beta-Poisson model for *E. coli* O157 parameters $\alpha = 8.4 \times 10^{-2}$, $N_{50} = 5.52 \times 10^{-3}$ (Teunis et al., 2004), cited in (CAMRA, 2019). Calculated $ID_{50} 5.6 \times 10^3$ for *E. coli* O157 in contrast 7.1×10^7 for *E. coli*.

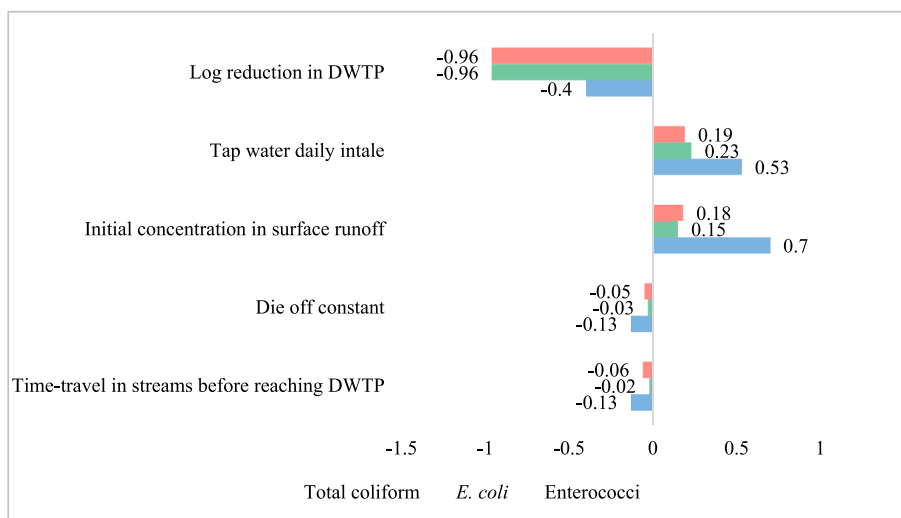


Fig. 4. Correlation coefficients of model parameters correspond to different FIBs.

3.5. Comparison with similar studies

It must be noted that the survival, growth and die of microorganisms following land application of animal waste is influenced by the composition of treatments, tillage practice, soil properties, and climatic factors. For example, Hill et al. (2005) reported that the predominant bacteria found in the experimental plots containing cattle sludge is *E. coli*. While findings indicate following guidelines of no application of wastes within 48 h of a prediction rain event, *E. coli* numbers in runoff may prove to be much higher when wastes are applied to tilled fields (Harrigan et al., 2004) as the greatest *E. coli* concentration in the runoff from the pasture site was observed from the no-till plots on day-3 after manure application. Furthermore, the *E. coli* level in the runoff was inversely proportional to tillage intensity (Harrigan et al., 2004).

Fenlon et al. (2000) investigated the fate of *E. coli* and *E. coli* O157 in cattle slurry after land application, and the results revealed that the soil texture class highly influences the reduction of microorganism count in the soil as a 5 log₁₀ reduction of *E. coli* O157 took 8 weeks and 25 weeks for sandy and clay soil, respectively. Blaustein et al. (2016) found that the rainfall intensity is positively correlated with the rainwater

partitioning to runoff. The rainfall intensity has significant and inverse effects on the numbers of bacteria remaining in the soil after rainfall. As rainfall intensity and soil profile depth increased, the numbers of microorganisms tended to decrease (Blaustein et al., 2016). A decrease in the number of bacteria colonies after the simulated rainfall events was reported in the literature, while periods of heavy rainfall may produce substantial losses of *E. coli* by both leaching and runoff (Fenlon et al., 2000; Hill et al., 2005). Also, critical rainfall probabilities can be multiplied with the overall microbial/pollutant risk assessment framework to calculate conditional probability and better estimate microbial risk through the drinking water pathway (Teng et al., 2012).

In another study (Guzman et al., 2010), no significant differences were reported in the average event mean concentrations (EMCs) of *E. coli* relative to storm intensity, while the statistically significant parameter for the average EMCs was the time lag between litter application and rainfall. The land application of animal waste increases the concentration of microorganisms in the runoff. However, a nonlinear relationship was observed between average *E. coli* EMC and time lag, with the EMC decreasing between 0 h (1.6×10^5 MPN 100 ml⁻¹) and 24 h (1.3×10^4 MPN 100 ml⁻¹) and then increasing at 120 h (4.3×10^4

MPN 100 ml⁻¹) showing only 0.57 log₁₀ most probable concentration of *E. coli* within 5 days (Guzman et al., 2010). Hubbs (2002) reported that 1–2 log₁₀ reduction of faecal coliform concentration in surface runoff from pastures with applied dairy manure could be achieved in 30 days. Furthermore, the competition among the many types of bacterial colonies found in the tilled plots influences the fate of the microorganisms following the land spreading of animal waste. Coliforms probably have to compete more with the soil microflora for available nutrients (Hill et al., 2005). These findings are in line with Nolan et al. (2020), on which the current risk assessment research is conducted.

3.6. Assumptions, limitations, and recommendations for future work

- i. The assumption was made that the edge of field runoff water may enter a nearby stream and be treated directly by a DWTP.
- ii. The losses determined using field rainfall simulators represent the worst-case loss scenario as these losses simulate the edge of field losses. Therefore, these losses do not account for potential attenuation, which may occur along the transfer continuum before reaching surface water (Nolan et al., 2020).
- iii. The default dilution factor was assumed as 10, which is a fixed value. The variability of this parameter was not captured, and so, it has not appeared in the sensitivity analysis outcome. This study proposes a future extension to assess time, intensity, discharge, and distance-dependant dilution factor, improving the current knowledge gap in this area.
- iv. One treatment process was considered in the DWTP; other configurations may exist and require separate assessment in terms of removal efficiencies. Future data collation in an Irish context (for Table 3) will minimise the uncertainties of this model.
- v. It was assumed that the entire population consumes tap water; however, many people consume bottled/package water in reality. Filtration processes such as reverse osmosis and ultraviolet treatments in homes are not included in the QMRA model.
- vi. Cross-contamination was not considered in this study, but it may be unavoidable in the distribution system and can play an important role in a similar QMRA.
- vii. As this study only focused on FIBs, future studies are needed to assess the fate of specific enteric pathogens.

4. Conclusions

Based on the probabilistic model, the highest daily human exposure (HE_{daily}) was observed for *E. coli* for treatment T1 on day 1 followed by TC for treatment T1 on day 1, *E. coli* for T1 on day 2, for T2 on day 1, and T2 on day 2. Out of 60 (20 × 3) cases, the simulated mean HE_{daily} exceeded 1 CFU day⁻¹ only in one case, which is *E. coli* (T1 & d 1). The overall simulated mean annual probability of illness (P_{annual}) due to *E. coli* is very low or low based on the WHO limit of 10⁻⁶ (1 per million people), representing a safe or no-risk limit. In one scenario, which is the application of raw slurry on day 1, resulting in a moderate risk being simulated mean P_{annual} as 1.0 × 10⁻⁵ (95th percentile 4.9 × 10⁻⁵), which is the worst-case scenario. Unpasteurised digestate (T2) showed low risk on days 1 and 2; however, there are very low numbers of FIBs in runoff associated with digestate after the 2nd day, and therefore, beyond day 2, there is very low risk associated with *E. coli* in surface runoff irrespective of treatment. Therefore, a restriction on applying bio-based fertiliser is important if there is any rain forecast within 48 h from the application time. The sensitivity analysis identified that TC and *E. coli* followed a similar pattern in terms of the importance of log reduction in drinking water treatment (negatively correlated) and daily intake of tap water (positively correlated), followed by initial concentration in the surface runoff (positively correlated). For enterococci, the most sensitive parameter was the initial concentration in the surface runoff, followed by daily intake of tap water (DITW) (both positively correlated) and log reduction in drinking water treatment (negatively correlated). Also, the

survival, growth and die of microorganisms following land application of animal waste are influenced by the composition of treatments, tillage practice, soil properties, and climatic factors. The risk assessment model developed in this study will help water managers in local authorities and regulatory agencies to evaluate the likely risk of *E. coli* entering potable water following bio-fertiliser application on agricultural land and help to improve the safe supply of drinking water.

CRedit author contribution statement

Rajat Nag: Conceptualisation, Methodology, Software, Data curation, Visualisation, Investigation, Writing - original draft preparation. **Stephen Nolan:** Conceptualisation, Investigation, Data curation. **Bryan K. Markey, Paul Whyte, Vincent O'Flaherty, Declan Bolton, Owen Fenton, Karl G. Richards:** Project management, Funding, Writing - reviewing and editing. **Enda Cummins:** Conceptualisation, Project management, Funding, Supervision, Writing - reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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