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Ranking hazards pertaining to human health concerns from land application of anaerobic digestate

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HIGHLIGHTS

• Health risks from spreading animal waste and/or anaerobic digestate.
• Semi-quantitative screening tool developed to rank pathogens.
• Scoring pathogens on thermal survivability, exposure pathways, severity or human mortality rate in untreated patients.

GRAPHICAL ABSTRACT

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ABSTRACT

Anaerobic digestion (AD) has been identified as one of the cleanest producers of green energy. AD typically uses organic materials as feedstock and, through a series of biological processes, produces methane. Farmyard manure and slurry (FYM&S) are important AD feedstock and are typically mixed with agricultural waste, grass and/or food wastes. The feedstock may contain many different pathogens which can survive the AD process and hence also possibly be present in the final digestate. In this study, a semi-quantitative screening tool was developed to rank pathogens of potential health concern emerging from AD digestate. A scoring system was used to categorise likely inactivation during AD, hazard pathways and finally, severity as determined from reported human mortality rates, number of global human deaths and infections per 100,000 populations. Five different conditions including mesophilic and thermophilic AD and three different pasteurisation conditions were assessed in terms of specific pathogen inactivation. In addition, a number of scenarios were assessed to consider foodborne incidence data from Ireland and Europe and to investigate the impact of raw FYM&S application (without AD and pasteurisation). A sensitivity analysis revealed that the score for the mortality rate (S3) was the most sensitive parameter (rank coefficient 0.49) to influence the final score S; followed by thermal inactivation score (S1, 0.25) and potential contamination pathways (S2, 0.16). Across all the scenarios considered, the screening tool prioritised Cryptosporidium parvum, Salmonella spp., norovirus, Streptococcus pyogenes, enteropathogenic E. coli (EPEC), Mycobacterium spp., Salmonella typhi (followed by S. paratyphi), Clostridium spp., Listeria.
1. Introduction

Harmful pathogens can be present in higher concentrations in animal FYM&S (Jones and Martin, 2003; Avery et al., 2004; Nicholson et al., 2005) compared to food waste (Jones and Martin, 2003), grass and agricultural residues (Seadi and Lukehurst, 2012). Hutchinson et al. (2004) reported high numbers of zoonotic pathogens (E. coli O157, Salmonella, Listeria monocytogenes, Campylobacter, Cryptosporidium parvum, Giardia intestinalis) in both fresh and stored animal waste (cattle, pig, poultry and sheep). The application of raw manure and slurry is standard practice on farms to utilise animal waste while also replenishing nutrients to the soil (Zoghi et al., 2015). AD is a process which can also use FYM&S as a feedstock and, by the action of microorganisms, break down biodegradable organic compounds into simpler molecules in the absence of oxygen to produce methane (Abbas et al., 2012; Manyi-Loh et al., 2013, 2016). The methane can also be cleaned and use as a fossil fuel replacement for transport and domestic use (Purdy et al., 2018). Another advantage of AD is that the process itself can inactivate pathogens; however, complete inactivation is not always achieved; for example, Smith et al. (2005) reported a 2 log reduction in E. coli could be achieved by mesophilic AD (M-AD). However, E. coli can be present as high as 6 log CFU g⁻¹ (Hutchison et al., 2004) in fresh cattle manure and therefore, there is the potential for E. coli to survive the M-AD process.

AD processes typically fall into three types (i) mesophilic (35 to 45°C) AD (or M-AD), (ii) thermophilic (45 to 80°C) AD (or T-AD) and (iii) two-step/phase AD; which is a combination of M-AD and T-AD (Sakar et al., 2009; Abbas et al., 2012; Manyi-Loh et al., 2013; Vanegas and Bartlett, 2013). M-AD is the most common system in Ireland (Smyth et al., 2009). It has a more stable operation but a lower biogas production rate compared to other types of AD. In contrast, the higher temperature process (T-AD) reduces pathogen numbers even further and provides more rapid reaction rates than M-AD (Mahmud et al., 2016). Process parameters such as temperature, pH, hydraulic retention time, organic loading rate, carbon-nitrogen ratio and free ammonia presence can also have a significant influence on pathogen inactivation (Sakar et al., 2009; Abbas et al., 2012; Manyi-Loh et al., 2013).

Waste-to-energy processes can play a role in the transition to a circular economy (European Commission, 2017). In the future, more consideration should be given to AD of biodegradable waste, where material recycling is combined with energy recovery (European Commission, 2017). Given the drive for renewable energy sources, the use of AD to process waste streams is likely to increase. There is a concern that several pathogens of significance may survive the process. Therefore, this study examined whether AD process residues (i.e. digestate) could re-enter the circular economy (Longhurst et al., 2019) by exploring issues of potential human, animal and environmental risk; and emphasises the considerable weight of evidence required to inform stakeholders of the safety of digestate.

Several additional methods can be used in conjunction with AD to reduce the number of pathogens in the final digestate. These include treatment with lime, chlorine, UV-light, ozone, high internal pressure in the vessel (Alvarez et al., 2003; Erickson and Ortega, 2006) or most commonly an additional heat treatment (pasteurisation) step (Smith et al., 2005). The European Commission recommends pasteurisation (heat treatment) at 70 °C for 1 h for feedstock before the AD process; whereas, there is a national transformative parameter recommendation of 60 °C for 48 continuous hours twice (DAFM, 2014) in Ireland. All these processes influence the level of pathogens in the final AD digestate, which is destined for application to agricultural land.

Several disease outbreaks have been observed in Europe over the last 20 years (Eurosurveillance, 2019) as highlighted in Fig. 1. It is understood that Salmonella, influenza virus, meases virus, Cryptosporidium and E. coli are the top five pathogens which have been responsible for several human health outbreaks in Europe; however, influenza virus and meases virus can only be transmitted from person to person (Waring et al., 2005; Li et al., 2009; Borges et al., 2016). In terms of the application of AD digestate to the agricultural land person to person is a non-critical pathway. Airborne, foodborne, waterborne and animal contact (zoonotic) diseases are of greatest human health concern (Health Service Executive, 2019). Foodborne illness (gastroenteritis) is a particular global health concern (WHO, 2008; Thomas et al., 2013; Torgerson et al., 2015). Nag et al. (2019) mentioned that the application of raw FYM&S and anaerobic digestate could possibly play a role in pathogen transportation from agricultural land to humans through the food chain (mainly ready to eat RTE crops). According to TIME Health (2017), 351,000 people die of food-poisoning globally every year. Foodborne disease means, according to WHO (2008), any disease of an infectious or toxic nature caused by consumption of food and a foodborne disease outbreak can be defined in the following ways,

a) The observed number of cases of disease exceeds the expected number
b) The occurrence of two or more cases of a similar foodborne disease resulting from the ingestion of a common food.

The Health Protection Surveillance Centre (HSE, 2019) cited by Nag et al. (2019) suggests that Clostridium, Cryptosporidium, E. coli, Salmonella are the main pathogens of human health concern in Ireland. This highlights the importance of considering the severity (fatality/mortality rate) rather than simply the number of confirmed cases in an outbreak. Tropical diseases; mostly parasites (helminths) and some viral diseases such as yellow fever virus, West Nile virus, dengue virus, tick-borne encephalitis virus, zika virus, ebola virus, lassa virus, marburg virus (Hotez et al., 2007) are not common in Ireland and there is no historical evidence of such outbreaks in Europe.

In some countries such as Denmark, animal manure is treated with mixed municipal sewage (Hartmann et al., 2002). Therefore, pathogens which are present both in animal manure, slurry and human effluent need to be considered in the European context. In contrast, grass, agricultural residues, animal manure and slurry, the organic fraction of municipal solid waste (comprises food and garden waste only) are considered the only feedstock used in AD plants in Ireland (Singh et al., 2010). The pathogens which have possible transmission pathways such as air, soil or food, water, and animal contact/zoonotic were considered for this study, while diseases which can be spread only by person-to-person contact (HPSC, 2005) or insect bites were excluded.

It is widely accepted as good practice in risk assessments to carry out an initial screening to identify hazards of greatest concern. There are two broad methods of risk assessment; qualitative and quantitative. When there are limited data a qualitative approach is recommended for decision making (Lammerding and Fazil, 2000). A semi-quantitative model is a bridge between qualitative and quantitative risk assessment models where risk factor categories are typically given a score and final risk scores calculated (Teunis and Schijven, 2019).
The principal hypothesis of this study was “Pathogens have a different propensity to survive the AD process while also potentially affecting humans through different pathways”. Hence, the overall aim of this study was to identify the key hazardous pathogens of potential human health concern in Europe and specifically in the Republic of Ireland which can be transmitted through FYM&S and anaerobic digestate using a semi-quantitative screening method.

2. Materials and methods

In this study, a semi-quantitative screening method was developed. A framework of the approach is given in Fig. 2. Five different time-temperature conditions such as M-AD 37 °C (4 days), T-AD 55 °C (4 days), Irish pasteurisation 60 °C (4 days), EU pasteurisation 70 °C (60 min), and higher pasteurisation 90 °C (60 min) were monitored (Table 1) for the baseline model (BM) to assess the likely fate of the pathogens after the AD process. As recommended by Nag et al. (2019) a semi-quantitative model was used in this study to rank the most hazardous pathogens depending on their ability to survive the AD process, while also potentially affecting the possible routes (aerosol, ingestion and direct contact) of transmission and the potential severity of illness. Indicator organisms are often used as surrogates for pathogens (Harwood et al., 2005). Table 2 shows the widely accepted indicator organisms for such studies. Assessing the ability of the process to inactivate indicator organisms should provide a high degree of confidence regarding inactivation of comparable pathogens.

2.1. Baseline model (BM)

As a primary qualitative/semi-quantitative screening process for risk assessment, the likelihood-severity (L × S matrix) approach has been used (Shariff and Zaini, 2013). The likelihood (L) of exposure to pathogens is influenced by two parameters in this model; the first one is the inactivation of pathogens (S1) through the AD process and secondly, the ability of pathogens spreading through different environmental pathways (S2) (such as air, soil attached to food, water or animal contact). The mortality rate (S3) was used to consider the likely severity for humans following infection by a particular pathogen.

2.1.1. Initial hazard selection

Using the scientific literature (Carrington, 2001; Jones and Martin, 2003; Lepeule et al., 2004; WHO, 2008; Longhurst et al., 2013; Torgerson et al., 2015) and the Eurosurveillance (2019) database, data from 300 outbreaks over the last 20 years were analysed (Fig. 2). This represents a broad list of hazards (Table S1 of the supplementary note) in the past which potentially represent a human health challenge. According to AFBI and DAFM (2019), gastrointestinal infection, respiratory infections, systemic infection, clostridial infection, cardiac and liver disease are the most common diseases in cattle. Whereas, sheep mortality is predominantly caused by parasitic diseases, respiratory infections, septicaemia, clostridial and enteric disease. Pneumonia, enteric infection, septicaemia and nervous system diseases are the predominant causes of pig mortality. Septicaemia, digestive, musculoskeletal, respiratory and parasitic diseases are common in the poultry industry. The relative frequency of pathogens found in post-mortem analysis on the carcass and faecal samples of dead animals are detailed in Table 3.

2.1.2. Influence of thermal treatment

The fate and inactivation of pathogens under different process conditions varies greatly (Table S2) which makes it difficult to compare their behaviour under standard process conditions detailed in Table 1. Hence, the Z value concept, which indicates the temperature rise necessary to reduce the decimal reduction time (‘D’ value) by one log10 (Juneja and Marmer, 1999; Bertolatti et al., 2001), was used to compare the inactivation conditions. Thermal inactivation data for each of the pathogens were collected from the available literature with a specific focus on the time-temperature relationship with Z value (reference temperature at which the time-temperature inactivation tests were done) and Dref (duration of heating at Tref for complete inactivation of the pathogen). Songer (2010) indicated that microbial inactivation
of spore-forming organisms is difficult as spores are much more heat resistant compared to the parent cells and spores can survive in the soil for many years (Sahlström, 2003). Therefore, the spore-forming criteria (Table S2) were considered in order to select suitable indicator bacteria.

For example, enterohemorrhagic E. coli O157: H7 can be inactivated at 55 °C for 40 min; Eq. (1) can investigate whether inactivation occurs at 37 °C (4 days), 55 °C (4 days), 60 °C (4 days), 70 °C (60 min), and 90 °C (60 min). Most of the references mentioned in Table S3 indicated a linear relationship between pathogen survival or inactivation and temperature at a shorter temperature range (35 °C to 90 °C). Hence, an appropriate temperature was adopted for the normalization process.

For another example, Salmonella enterica spp can be inactivated by heating at 60 °C for 60 mins or 121 °C for 15 min (Table S2); hence, the lower temperature-time (60 °C for 60 mins) was adopted for calculation. Similarly, enterohemorrhagic E. coli O157: H7 can be inactivated by 55 °C for 40 min or 45 °C for 24 h (Table S2); therefore, 55 °C for 40 min was adopted for the inactivation reference as it is closer to the mean temperature (62.4 °C) of comparable scenarios (Table 1).

\[
\text{New } D_{\text{value min}}(\text{T}) = \frac{D_{\text{ref}}}{C_{138}^{Z(\text{T})}} = \frac{D_{\text{ref}}}{C_{138}^{Z(\text{T})}}(1) 
\]

where,

**Table 1**

| Number | Name         | Description       | Time | Temperature |
|--------|--------------|-------------------|------|-------------|
| 1      | M-AD         | Mesophilic AD     | 4 days | 37 °C       |
| 2      | T-AD         | Thermophilic AD   | 4 days | 55 °C       |
| 3      | Pas 1        | Irish pasteurisation | 4 days | 60 °C       |
| 4      | Pas 2        | EU pasteurisation | 60 min | 70 °C       |
| 5      | Pas 3        | Higher pasteurisation | 60 min | 90 °C       |

Fig. 2. Flow diagram of the screening method.
\[ T_{\text{ref}} \, (°C) = \text{reference temperature from the literature at which the time-temperature inactivation tests were done}; \text{ for enterohemorrhagic } E. \text{coli O157: H7 example, say 55 °C (from Table S2)}. \]

\[ D_{\text{ref}} \, (\text{min}) = \text{duration of heating at } T_{\text{ref}} \text{ for the experiment considering complete inactivation of the pathogen}; \text{ for the above example, say 40 min (from Table S2)}. \]

\[ Z_{\text{value}} \, (°C) = \text{temperature rise necessary to reduce decimal reduction time by one logarithmic cycle}; \text{ for the above example, a value of 9.15 °C is used which is the average from two studies considered which give a } Z_{\text{value}} \text{ of 6 °C and 12.3 °C for reference temperatures 65 °C and 50 to 70 °C, respectively. (Table S2)}. \]

\[ T_{\text{new}} \, (°C) = 37 °C \text{ (mesophilic condition)} \]

\[ \text{New } D_{\text{value}} \, (\text{min}) \text{ (for mesophilic condition)} = \frac{40}{10((37 – 55)/9.15)} = 3709 \text{ min (2.57 days). This New } D_{\text{value}} \text{ is used to score (S1) pathogens (Eq. (2)).} \]

\[ \text{Similarly, new } D_{\text{value}} \, (\text{min}) \text{ for thermophilic conditions (55 °C) was 40 min (0.027 days); and for three pasteurisation conditions (60 °C, 70 °C, 90 °C) it was calculated as 11.36 min, 0.917 min and 0.006 min, respectively. Hence, the bacteria could be fully inactivated through all AD and pasteurisation conditions. There are a lot of studies carried out using bacteria; however, there are gaps in the literature for fungi,} \]

### Table 2

List of commonly used indicator pathogens.

| Name                     | Indicator for                                      | Reference                                      |
|--------------------------|----------------------------------------------------|------------------------------------------------|
| Escherichia coli         | Gram –ve, non-spore forming coliform bacteria      | (Johansson et al., 2005)                      |
| Salmonella senftenberg   | Gram –ve, non-spore forming bacteria               | (Wheeler et al., 1943; Mocé-illivina, 2003)   |
| Enterococcus faecalis    | Gram + ve, non-spore forming bacteria              | (McFeters et al., 1974; Mocé-illivina, 2003; Sahlström, 2003; Anderson et al., 2005; Sidhu and Toze, 2009) |
| Clostridium sp.          | Gram +ve, spore-forming bacteria                   | (Payment and Franco, 1993; Ferguson et al., 1996; Fewtrell and Bartram, 2001) |
| Mycobacterium sp.        | Acid-fast thermoresistant bacteria                 | (Deb et al., 2009)                            |
| Feline calicivirus (FCV) | Virus. Non-envelope virus; more heat resistant. Enteric virus (gene levels of noroviruses) | (Wong et al., 2010; Cook, 2013; Cromeans et al., 2014) |
| Cryptosporidium parvum   | Parasites                                          | (Harwood et al., 2005)                        |

### Table 3

Animal diseases found in Ireland and typical symptoms. (Source: DAFM).

| Diseases                          | Pathogens                                                                 | Relative frequency of population deaths (%) in 2016 |
|-----------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------|
| Cattle                            |                                                                           |                                                    |
| Gastrointestinal infection (Enteritis and Parasitic) | Bovine Diarrhoeal Virus, Salmonella, Liver fluke, Rumen fluke, gut worms (stomach and intestinal) | 12                                                  |
| Respiratory infections (pneumonia, pleuroneumonia and parasitic bronchitis) | Mycobacterium, Bovine respiratory syncytial virus (RSV), Trueperella pyogenes, Mannheimia haemolytica, Dicytocalus spp., Mycoplasma bovis, Pasteurella multocida, bovine herpesvirus, Histophilus somni | 17                                                  |
| Systemic infection                |                                                                           |                                                    |
| Clostridial infection             |                                                                           |                                                    |
| Cardiac infection                 |                                                                           |                                                    |
| Liver disease                     |                                                                           |                                                    |
| Bovine abortion                   |                                                                           |                                                    |
| Bovine mastitis                   |                                                                           |                                                    |
| Sheep                             |                                                                           |                                                    |
| Parasitic disease                 |                                                                           |                                                    |
| Respiratory infections            |                                                                           |                                                    |
| Septicaemia                       |                                                                           |                                                    |
| Clostridial and Kidney disease    |                                                                           |                                                    |
| Enteric disease                   |                                                                           |                                                    |
| Ovine abortion                    |                                                                           |                                                    |
| Pig                               |                                                                           |                                                    |
| Pneumonia                         |                                                                           |                                                    |
| Colibacillosis and Enteric infection |                                                                           |                                                    |
| Septicaemia                       |                                                                           |                                                    |
| Nervous disease                   |                                                                           |                                                    |
| Poultry                           |                                                                           |                                                    |
| Septicaemia                       |                                                                           |                                                    |
| Digestive                         |                                                                           |                                                    |
| Musculoskeletal                   |                                                                           |                                                    |
| Respiratory                       |                                                                           |                                                    |
| Parasitic disease                 |                                                                           |                                                    |

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parasites and some of the viruses. This is reflected in Table S2 as the ‘Z’ value for all fungi, parasites and viruses was not available. Bozkurt et al. (2014) recommended the ‘Z’ value for hepatitis A virus as 14.43 °C which was adopted for all viruses in the absence of data. The entire calculation for 91 pathogens is presented in Table S4.

2.1.3. Screening strategy

A screening score was incorporated depending on the inactivation rate ($S_1$) of the pathogen through the thermal process comparing the process duration (Table 1) and time required for full inactivation of the target pathogen (Fig. 2).

If the calculated ‘New Dvalue’ is lower than the process duration mentioned in Table 1, $S_1$ is set to 0.001 otherwise,

$$S_1 = \frac{\text{New Dvalue} - \text{process duration}}{\text{New Dvalue}}$$ (2)

Bio-aerosols, water, ingestion of soil through food and direct contact with infected animals were identified as major hazard pathways and the main pathogens which are typically transmitted through those four pathways were identified from the literature (Ashbolt, 2004; Thomas et al., 2013; Arfken et al., 2015; Klous et al., 2016; Van Leuken et al., 2016; Conrad et al., 2017). Score $S_2$ was given (Table S5) according to their transmission likelihood ($L$). If a pathogen can travel through four media such as air, soil or food, water, and animal contact it achieved the highest accumulated score of 1 (0.25 for each pathway; for example, Cryptosporidium parvum). Otherwise, a score of 0.25 was given for each pathway (Fig. 2).

The mortality rate was selected to consider the severity on human health following infection; the score $S_3$ represents the mortality rate from 0.1 to 1 where 0.1 stands for 10% and 1 for 100% mortality in untreated patients. In the absence of a mortality rate, the score was proposed based on the number of annual human deaths globally (Fig. 3) where 0 to 100 deaths were assigned a low score and >10,000 deaths corresponded to a high score. Infection or illness cases per 100,000 population was another alternative approach as mortality rate and global deaths due to all 91 pathogens was not available. A low score was given where infection or illness was <1 per 100,000 population; the value between 1 and 99 was assigned a moderate score; and, a high score was given to 100 or more incidents per 100,000 population (Fig. 3). If any of these three criteria were not fulfilled, a low score (0.3) was given for the consistency of the model (Fig. 3). This step was introduced to consider the ‘severity’ of the hazard within likelihood-severity ($L \times S$) matrix. The final score $S$ of the screening process was based on the multiplication of three individual scores $S_1$, $S_2$ and $S_3$ (Eq. (3)). The scores were multiplied so the absence of any one score will result in the elimination of risk.

$$S = S_1 \times S_2 \times S_3$$ (3)

2.1.4. Comparison with indicator organisms

In this part of the study, pathogens with the highest scores were cross-checked with the indicator pathogens. Pathogens were categorised mainly as bacteria, parasites and viruses. During this investigation, the authors considered parameters such as; mortality rate, host and reservoirs of pathogens, identification of vectors (secondary

![Fig. 3. Adopted strategy for S3 scoring.](image)
| Number | Pathogens                  | Number of confirmed human cases in Ireland | Total number of confirmed cases/100,000 population (notification rates) | Avg. value | Score S3 Ire |
|--------|---------------------------|-------------------------------------------|------------------------------------------------------------------------|------------|--------------|
| 1      | Campylobacter spp.        | 2511 2453 2983 2288 2391 2433 1800 1810 1752 1885 | 52.1 53 56.3 49.8 52.17 54.3 37.15 40.67 39.8 43.7 47.99 | 0.9        |              |
| 2      | Salmonella spp.           | 299 270 259 326 309 311 349 335 447 440   | 6.3 5.8 5.6 7.1 6.7 6.9 7.8 7.5 10.2 10.2 7.41 | 0.8        |              |
| 3      | Yersinia spp.             | 3 13 5 4 2 6 3 3 6 0.06 0.28 0.11 0.09 0.04 0.13 0.07 0.07 0.1 0.1 0.105 0.7 |              |
| 4      | E. coli                   | 737 598 572 564 412 275 197 237 213 115 | 15.6 12.92 12.42 12.29 8.99 6.14 4.41 5.33 4.8 2.7 8.56 0.8 |              |
| 5      | Listeria monocytogenes    | 13 19 15 8 11 7 10 10 13 21 0.28 0.41 0.33 0.17 0.24 0.16 0.22 0.22 0.3 0.5 0.283 0.7 |              |
| 6      | Coxiella burnetii         | 6 4 0 5 9 17 0.13 0.09 0.11 0.2 0.4 0.132 0.7 |              |
| 7      | Echinococcus spp.         | 2 0 0 1 1 0 1 1 2 0.04 0 0.02 0.02 0 0.02 0.02 0 0 0.012 0.6 |              |
| 8      | Brucella spp.             | 2 0 3 1 2 1 1 0 2 0.04 0 0.07 0.02 0.04 0.02 |              |
| 9      | Trichinella spp.          | 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 |              |
| 10     | Mycobacterium spp.        | 3 5 3 6 4 6 7 11 5 5 0.06 0.11 0.07 0.13 0.08 0.13 |              |
| 11     | Toxoplasma gondii         | 0 1 0 1 1 1 37 0 1.5 0 1.5 1.4 1.36 0.83 |              |
| 12     | Vibrio spp.               |                                           |              |
| 13     | Clostridium spp.          |                                           |              |
| 14     | Norovirus                 | 50 28 1.1 0.616 0.858 0.7 |              |
| 15     | Hepatitis A               |                                           |              |
| 16     | Cryptosporidium           | 439 394 514 556 428 294 445 416 609 10.38 9.31 12.15 13.14 10.12 6.95 10.52 9.83 14.4 10.75 |              |

* Scale for selecting score S3 Ire based on the total number of confirmed cases/100,000 population (notification rates).
* Blank cells represent unavailability of data in the report.
* Only Cryptosporidium data has been collected from The Health Protection Surveillance Centre (HPSC) (2018).
* Number of confirmed cases/100,000 population range Score S3 Ire
  * 100 10 0.9
  * 9.9 1 0.8
  * 0.99 0.1 0.7
  * 0.099 0.01 0.6
  * 0.0099 0.001 0.5
  * 0.00099 0.0001 0.4
Table 6
Pathogens considered for Scenario BFOODEU.

| Number | Pathogens                  | Number of confirmed human cases in the EUb,c | Total number of confirmed cases/100,000 population (notification rates)b,c,d | Avg. value | Score S3EU |
|--------|----------------------------|---------------------------------------------|---------------------------------------------------------------------------------|-----------|------------|
|        |                            | 2016 | 2015 | 2014 | 2013 | 2012 | 2011 | 2010 | 2009 | 2008 | 2007 | 2016 | 2015 | 2014 | 2013 | 2012 | 2011 | 2010 | 2009 | 2008 | 2007 |
| 1      | Campylobacter spp.         | 246,307 | 232,134 | 236,818 | 214,710 | 214,300 | 220,209 | 215,397 | 198,725 | 190,579 | 200,980 | 66.3 | 62.9 | 66.5 | 61.4 | 61.7 | 50.28 | 48.56 | 45.57 | 40.7 | 45.2 | 54.91 | 0.9 |
| 2      | Salmonella spp.            | 94,530 | 94,597 | 92,012 | 87,753 | 95,548 | 101,037 | 110,181 | 83,56 | 88,03 | 18.4 | 20.9 | 20.7 | 20.3 | 21.9 | 20.7 | 21.5 | 24 | 26.4 | 31.1 | 22.7 | 0.9 |
| 3      | Yersinia spp.              | 6861 | 6928 | 6352 | 6215 | 7017 | 6780 | 7578 | 8356 | 8803 | 1.82 | 1.91 | 1.83 | 1.92 | 1.93 | 1.63 | 1.58 | 1.65 | 1.8 | 2.8 | 1.88 | 0.8 |
| 4      | E. coli                    | 6378 | 5929 | 5900 | 6042 | 5680 | 9485 | 3656 | 3583 | 3159 | 3271 | 1.82 | 1.68 | 1.75 | 1.8 | 1.7 | 1.93 | 0.83 | 0.75 | 0.7 | 0.6 | 1.35 | 0.8 |
| 5      | Listeria monocytogenes     | 2336 | 2206 | 2242 | 1883 | 1720 | 1601 | 1654 | 1425 | 1581 | 0.47 | 0.43 | 0.46 | 0.39 | 0.36 | 0.32 | 0.35 | 0.36 | 0.3 | 0.3 | 0.37 | 0.7 |
| 6      | Coxiella burnetii          | 1057 | 822 | 780 | 647 | 518 | 1414 | 1988 | 1660 | 605 | 0.16 | 0.18 | 0.18 | 0.15 | 0.12 | 0.36 | 0.51 | 0.5 | 0.27 | 0.7 |
| 7      | Echinococcus spp.          | 772 | 883 | 820 | 805 | 865 | 781 | 756 | 775 | 909 | 972 | 0.2 | 0.2 | 0.19 | 0.18 | 0.2 | 0.18 | 0.16 | 0.18 | 0.2 | 0.2 | 0.18 | 0.7 |
| 8      | Brucella spp.              | 516 | 437 | 462 | 498 | 501 | 330 | 356 | 404 | 735 | 639 | 0.12 | 0.09 | 0.09 | 0.1 | 0.1 | 0.07 | 0.07 | 0.08 | 0.1 | 0.1 | 0.1 | 0.1 | 0.09 | 0.6 |
| 9      | Trichinella spp.           | 101 | 156 | 324 | 217 | 301 | 268 | 223 | 750 | 670 | 787 | 0.02 | 0.03 | 0.06 | 0.04 | 0.06 | 0.05 | 0.05 | 0.16 | 0.1 | 0.2 | 0.077 | 0.6 |
| 10     | Mycobacterium spp.         | 170 | 181 | 167 | 144 | 132 | 132 | 165 | 134 | 123 | 113 | 0.04 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | 0.01 | 0.031 | 0.6 |
| 11     | Toxoplasma gondii          | 47 | 288 | 258 | 213 | 144 | 21 | 289 | 11 | 16 | 1.57 | 8.27 | 7.4 | 6.2 | 4.2 | 0.56 | 0.65 | 0.08 | 0.09 | 0.09 | 0.09 | 0.09 | 0.61 | 0.8 |
| 12     | Vibrio spp.                | 76 | 29 | 17 | -0.01 | -0.01 | 0.009 | 0.5 |
| 13     | Clostridium spp.           | 49 | 60 | 1727 | 2009 | 1729 | 1059 | 795 | 1704 | 857 | 0.01 | 0.01 | 0.04 | 0.06 | 0.03 | 0.03 | 0.02 | 0.03 | 0.03 | 0.02 | 0.03 | 0.02 | 0.08 | 0.6 |
| 14     | Norovirus                  | 11,993 | 13,536 | 3580 | 2023 | 13,987 | 2529 | 6533 | 2670 | 3617 | 0.08 | 0.06 | 0.06 | 0.02 | 0.23 | 0.123 | 0.7 |
| 15     | Hepatitis A                | 155 | 78 | 48 | 1444 | 116 | 7 | 13 | 2 | 104 | -0.01 | -0.01 | -0.01 | 0.005 | 0.5 |
| 16     | Cryptosporidium            | 62 | 120 | 24 | 65 | 11 | 20,000 | 12,700 | 87 | -0.01 | -0.01 | -0.01 | 0.009 | 0.5 |

a Scale for selecting score S3EU based on the total number of confirmed cases/100,000 population (notification rates).
b Iceland, Norway, Switzerland are excluded; no special agreement for data.
c Blank cells represent unavailability of data in the report.
d Number of confirmed cases/100,000 population range Score S3EU

100 | 0.9 | 0.9 |
9.9 | 1 | 0.8 |
0.99 | 0.1 | 0.7 |
0.999 | 0.01 | 0.6 |
0.0099 | 0.001 | 0.5 |
0.00099 | 0.0001 | 0.4 |
Fig. 4. The result of the screening model with five different conditions (BM).
2.2. Scenarios

Three scenarios were considered, scenario 1 was based on Ireland where pathogens associated with foodborne outbreaks in that country only were evaluated. In scenario 2 pathogens associated with any foodborne outbreak across Europe were incorporated into the model (Table 4). Scenario 3 looked at the situation where there is no AD inactivation or pasteurisation (S1 = 0), which can be considered as representative of the application of raw FYM&S on to land.

2.2.1. Scenario 1: model considering only foodborne illness in Ireland (Scenario AFOODIRE)

The methodology for Scenario AFOODIRE is similar to the BM; the only alteration was made in the S2 score. Instead of four pathways (air, soil or food, water, and animal contact), only the foodborne (including drinking water) pathway was considered (Table 4). Drinking water was considered as it is sometimes considered as a part of the food chain. However, ‘waterborne’ includes vast possibilities such as washing, swimming, drinking (with or without food), game/sports activity etc. (O’Flaherty and Cummins, 2017). The total number of confirmed cases/100,000 population in Ireland was determined (Table 5) and the same data source (EFSA reports) was used for this scenario (Table 4). The relative score S3IRE (ranging from 0.4 to 0.9; note of Table 5) was given depending on the ‘confirmed cases/100,000 population’ range (note, Table 5 referred). Similarly to Eq. (3), the final score (S) was calculated as $S_{1} \times S_{2} \times S_{3IRE}$.

2.2.2. Scenario 2: model considering only foodborne illness in Europe (Scenario BFOODEU)

Comparing to the Scenario AFOODIRE model, an alteration was made to check the scenario in Europe. Hence, the total number of confirmed cases/100,000 population in Europe was determined (Table 6) and the same data source (EFSA reports) was used for this scenario (Table 4). The relative score S3EU (ranging from 0.4 to 0.9; note of Table 6) was given depending on the ‘confirmed cases/100,000 population’ range (note, Table 6 referred). Similarly to Eq. (3), the final score (S) was calculated as $S_{1} \times S_{2} \times S_{3EU}$.

2.2.3. Scenario 3: model considering raw manure and slurry application without heat treatment and AD (Scenario CRAWFYM&S)

In this scenario (Scenario CRAWFYM&S), a comparison was made between the digested and raw manure and slurry. This scenario looked at the fate of pathogens if no anaerobic digestion and pasteurisation were used on the pathogens. The S1 score has no influence in this regard as compared to the BM; which means the final score (S) was calculated as S2 multiplied by S3 only (Table 4).

3. Results

3.1. Scores S1, S2, and, S3

The list of pathogens and their susceptible host species, source, mortality information, available outbreak data are tabulated in Table S1. Table S3 highlights the various factors affecting survival (aerobic/anaerobic), classification types (Gram-positive/negative), sporforming potential, time-temperature condition for heat inactivation and incubation period (the period over which eggs, cells, etc. are incubated). Depending on these criteria, the appropriate indicators for the pathogens were assigned to check when indicator pathogens are inactivated through the process and assess the potential of survival of the top-ranked pathogens.
Table 7
List of top scored pathogens from screening method and comparison with the indicator pathogens (baseline model BM).

| Number | Name                          | Type                                      | Indicator                        |
|--------|-------------------------------|-------------------------------------------|----------------------------------|
| 1      | Cryptosporidium parvum       | Parasites: Protozoa                       | Itself                           |
| 2      | Streptococcus pyogenes        | Gram +ve, aerobe, non-spore forming, non-coliform bacteria | Clostridium                      |
| 3      | Entamoeba histolytica         | Parasites: Protozoa                       | Itself                           |
| 4      | Salmonella enterica spp.     | Gram –ve, facultative anaerobe, non-spore forming, coliform bacteria | Cryptosporidium                  |
| 5      | Ascaris spp.                  | Parasites: helminths                      | Itself                           |
| 6      | E. coli enteropathogenic (EPEC) | Gram –ve, facultative anaerobe, non-spore forming coliform bacteria | Cryptosporidium                  |
| 7      | Mycobacterium spp.           | Acid-fast thermoresistant bacteria        | Itself                           |
| 8      | Salmonella typhi followed by S. paratyphi | Gram –ve, facultative anaerobe, non-spore forming, coliform bacteria | Itself                           |
| 9      | Giardia lamblia, Giardia intestinalis | Parasites: Protozoa                         | Cryptosporidium                  |
| 10     | Shigella spp.                 | Virus                                      | E. coli, Salmonella senftenberg   |
| 11     | Norovirus (surrogated by FCV) | Gram –ve, facultative anaerobe, non-spore forming, coliform bacteria | Itself                           |
| 12     | Enterobacter spp.             | Gram –ve, facultative anaerobe, non-spore forming, coliform bacteria | E. coli, Salmonella senftenberg   |
| 13     | Clostridium spp.              | Gram +ve, spore-forming bacteria          | Itself                           |
| 14     | Listeria monocytogenes        | Gram +ve, facultative anaerobe, non-spore forming, non-coliform bacteria | Itself/Enterococcus faecalis     |

4. Discussion

4.1. Most hazardous pathogens (primary observation)

Comparing the pathogens listed in Table 3 and S1 it can be concluded that pathogens such as Mycobacterium spp., Salmonella enterica spp., Listeria monocytogenes, Enterobacter spp., Clostridium spp. and E. coli are common both in human and animals. The common top-ranked pathogens which appeared in the BM (Fig. 5), Scenario A_foodire (Fig. 7a), Scenario B_foodieu (Fig. 7b), and Scenario C_RAWPYMS (Fig. 7c) models are Cryptosporidium parvum, Salmonella enterica spp., norovirus, Streptococcus pyogenes, Entamoeba histolytica, enteropathogenic E. coli (EPEC), Mycobacterium spp., Salmonella typhi followed by S. paratyphi, Clostridium spp., Listeria monocytogenes and Campylobacter coli. A comparison between results of A_foodire (Fig. 7a) and Scenario B_foodieu (Fig. 7b) highlights the difference between foodborne pathogens in Ireland and those found in the EU, with Cryptosporidium being noted as a greater issue in Ireland. According to the Health Protection Surveillance Centre (HPSC) (2018), there have been 400 to 600 cases (yearly) of cryptosporidiosis in Ireland since 2004. In the last scenario (Scenario C_RAWPYMS), no heat treatment was applied in terms of AD or pasteurisation; the additional pathogens of concern were Campylobacter jejuni, Vibrio spp., hepatitis A-virus, E. coli O157:H7, E. coli invasive & toxigenic, Streptococcus pneumoniae and rotavirus. A comparison of Fig. 5 and 7c highlights the effect of M-AD in reducing the final risk score for Salmonella typhi (and S. paratyphi) and norovirus. Other pathogens remained unchanged in terms of the ranking score; such as Cryptosporidium parvum, Streptococcus pyogenes, Entamoeba histolytica and Salmonella enterica spp. highlighting their heat resistance.

4.2. Sensitivity analysis

A sensitivity analysis was performed to find out the contribution of three scores S1, S2, and S3 to the final score S. The baseline model (BM) was used for sensitivity analysis (based on the top 14 pathogens). The correlation coefficient (Spearman rank) of three different scores S1, S2 and S3 were found as 0.25, 0.16 and 0.49, respectively (Fig. 8). Fig. 8 represents a systematic evaluation of the influencing parameters on the final risk score. The bars extending to the right-hand side indicate a positive correlation between these model inputs and the final risk score. Consequently, the score due to the mortality rate (S3) was identified as the most sensitive parameter of the model followed by thermal inactivation (S1) and score for potential contamination pathways (S2). Again, in some pathogens, the final score (S) which was presented in the form of bars, could be visible only in mesophilic conditions.
Therefore, it reinforces the influence of the inactivation score (Smith et al., 2005) on this screening method.

### 4.3. Comparison with indicator pathogens

A comparison with indicator pathogens (Table 7) gave confidence as out of seven indicators (Table 2), six matched (except Enterococcus faecalis) with the top 14 screened pathogens. Enterococcus faecalis is an opportunistic pathogen which generally affects elderly patients with underlying disease and other immunocompromised patients who have been hospitalized for long periods (Public Health Agency of Canada, 2019). According to Oprea and Zervos (2007), Enterococci are not classic foodborne pathogens. There are some animal pathogens other than those which are mentioned in Table S1 (AFBI and DAFM, 2016). A list of pathogens (other than Table S1) causing disease in animals and not in humans are presented in Table 8. The model can also

![Diagram](image-url)
be used to assess the pathogens of an animal health concern as a comparison between these pathogens and indicators used in the model can be readily carried out. In the absence of detailed thermal inactivation data (Tref, Dvalue and Zvalue), only a comparison was made to find out the indicators (final column of Table 8) and it is noted all indicators were already captured in this model (Table 7). Feline calicivirus (FCV), which is a non-enveloped virus, is a more heat resistant enteric virus (used as a surrogate for noroviruses) and generally causes illness in cats (Wong et al., 2010; Cook, 2013; Cromeans et al., 2014). However, it was not considered directly in the list of 91 pathogens as it is not likely to add a cat-carcass in an AD plant in Ireland. Finally, the choice of an indicator is very important and this can be limited to case-specific scenarios; for example, Cryptosporidium is a good indicator of parasites (matured cells); however, Ascaris eggs were found to be more resilient (Kato et al., 2004) compared with Cryptosporidium oocysts at all sampling points.

**Fig. 8.** The correlation coefficient (Spearman rank) of three different scores S1, S2 and S3 for the top 14 pathogens (BM).

**Table 8** List of pathogens (other than which are mentioned in Table S1) potentially representing an animal hazard (animal only, not human) and comparison with the indicators (AFBI and DAFM, 2016).

| Number | Pathogen name/cause | Name of hazard | Classification | Affected animals | Indicator | Cattle | Sheep | Pig | Poultry |
|--------|---------------------|----------------|----------------|-----------------|-----------|--------|-------|-----|---------|
| 1      | Actinobacillus pleuropneumoniae | Porcine pleuropneumonia | Gram-negative, facultative anaerobic bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | ✓ | |
| 2      | African Swine Fever virus (ASFV)* | African Swine Fever (ASF) | Virus | ✓ | Feline calicivirus (FCV) | |
| 3      | Babesia spp. | Babesiosis/tick-borne disease | Protozoa parasite | ✓ | Cryptosporidium parvum | |
| 4      | Bibersteinia trehalosi | Pneumonia | Gram-negative, facultative anaerobic bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | |
| 5      | Bluetongue virus | Bluetongue Disease (BT) | Virus | ✓ | Feline calicivirus (FCV) | |
| 6      | Bordetella bronchiseptica | Infectious bronchitis | Gram-negative, rod-shaped bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | ✓ | |
| 7      | Bovine Respiratory Syncytial virus | Respiratory disease | Virus | ✓ | Feline calicivirus (FCV) | |
| 8      | Brachyspira spp. | Diarrheal disease | Gram-negative, anaerobic bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | ✓ | |
| 9      | Chlamydia abortus | Abortive and fetal death in mammals | Gram-negative bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | ✓ | |
| 10     | Circovirus 2 | Affecting liver, lung etc. | Virus | ✓ | Feline calicivirus (FCV) | ✓ | |
| 11     | Coccidioden protozoa | Parasitic/Coccidiosis | Protozoa | ✓ | Cryptosporidium parvum | ✓ | |
| 12     | Dermanyssus gallinae | Affecting production and hen health | Parasites: Red mite, Arthropoda | ✓ | Cryptosporidium parvum | ✓ | |
| 13     | Dictyocaulus viviparus | Parasitic pneumonia | Parasites: helminths | ✓ | Ascaris/Cryptosporidium parvum | ✓ | |
| 14     | Echinocystoma spp. | Paramphistomiasis | Parasites: helminths | ✓ | Ascaris/Cryptosporidium parvum | ✓ | |
| 15     | Eimeria spp. | Coccidiosis | Protozoa parasite | ✓ | Cryptosporidium parvum | ✓ | |
| 16     | Erysipelotrix rhusiopathiae* | Erysipelas | Gram-positive, facultative anaerobic bacteria | ✓ | Enterococcus faecalis | ✓ | |
| 17     | Fasciola spp./Liver fluke | Chronic fasciolosis | Parasites: helminths | ✓ | Ascaris/Cryptosporidium parvum | ✓ | |
| 18     | Herpesvirus | Neoplasia/Marek’s disease | Virus | ✓ | Feline calicivirus (FCV) | ✓ | |
| 19     | Histophilus somni | Bovine respiratory disease | Gram-negative, facultative anaerobic bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | ✓ | |
| 20     | Mannheimia haemolytica | Respiratory disease | Gram-negative, anaerobic bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | ✓ | |
| 21     | Mycoplasma spp. | Pneumonia | Gram-positive bacteria | ✓ | Clostridium/Enterococcus faecalis | ✓ | |
| 22     | Nematode (Roundworms) | Parasitic gastroenteritis | Parasites: helminths | ✓ | Ascaris/Cryptosporidium parvum | ✓ | |
| 23     | Newcastle Disease virus* | Newcastle Disease Septicaemia | Virus | ✓ | Feline calicivirus (FCV) | ✓ | |
| 24     | Pasteurella spp. | Septicaemia | Gram-negative, facultative anaerobic bacteria | ✓ | Escherichia coli/Salmonella enterica spp. | ✓ | |
| 25     | Retrovirus* | Enzootic Bovine Leukosis (EBL) | Virus | ✓ | Feline calicivirus (FCV) | ✓ | |
| 26     | Rumen fluke | Liver fluke disease | Parasites: helminths | ✓ | Ascaris/Cryptosporidium parvum | ✓ | |
| 27     | Trueperella pyogenes | Abscesses, mastitis, metritis, and pneumonia | Gram-positive, facultative anaerobic bacteria | ✓ | Enterococcus faecalis | ✓ | |

* The health status of animals on the island of Ireland benefits from our island status and the geographical buffer provided by Great Britain and Western Europe.

**Zoonotic.**
Table 9 lists the pathogens (parasites) such as *Ascaris*, *Ancylostoma duodenale*, *Toxocara spp.*, *Entamoeba histolytica*, *Echinococcus multilocularis* and *Echinococcus granulosus* and the likely levels in urban wastewater and hospital waste; the presence of these pathogens in FYM&S is rare. It is not recommended to mix urban wastewater with FYM&S in an AD plant, hence limiting the likely presence of these parasites. Finally, this study looked to identify the top-ranked pathogens comparing common pathogens found in different scenarios such as BM, Scenario A FOODIRE (or Scenario B FOODEU) and Scenario C RAWFYM&S (Table 10). Table 10 provides a prioritisation of the highest-ranking pathogens likely to be of concern and requiring vigilance. The pathogens which appeared more than once in the scenarios (Table 10) are *Cryptosporidium parvum*, *Salmonella enterica* spp., *norovirus*, *Streptococcus pyogenes*, *Entamoeba histolytica*, *E. coli* enteropathogenic (EPEC), *Mycobacterium* spp., *Salmonella* typhi followed by *S. paratyphi*, *Clostridium* spp., *Listeria monocytogenes* and *Campylobacter coli* (11 in total). In

| Pathogen name | Likely levels | Unit | Source | Reference |
|---------------|---------------|------|--------|-----------|
| Ascaris spp.  | 0.7 to 13.33  | eggs l⁻¹ | Wastewater | (Amahmid et al., 1999) |
|               | 10.08 to 24.36 |      | Urban raw wastewater | (Maya et al., 2006; Hatam-Nahavandi et al., 2015) |
|               | 1344 to 4116  |      | Animal wastewater | (Anderson and Schad, 1985) |
| Ancylostoma duodenale | 100–150 | eggs g⁻¹ | Affected human stool | (Reynoldson et al., 1997) |
| Toxocara spp. | 0–4.35 | eggs g⁻¹ | Sand sample contaminated with faeces | (Uga, 1993) |
|               | mean 4.24 ± 4.62 and median 2.17 ± 5.92 | | Hair sample of contaminated dogs | (Devoy Keegan and Holland, 2010) |
| Trichinella spp. | 2 to 295 | larvae g⁻¹ | Contaminated meat | (Teunis et al., 2012) |
| Entamoeba histolytica | 2.5 × 10² to 5.0 × 10² | cysts l⁻¹ | Wastewater treatment plant influent | (Sabbahi et al., 2018) |
| Echinococcus multilocularis | 20–140 | cysts g⁻¹ | Faecal sample collected from infected patients in hospitals | (Yospawoe, 2016) |
| Echinococcus granulosus | 20–140 | eggs g⁻¹ | Faecal sample of infected dog; mostly red fox and racoon dogs; very rare disease in Europe | (Allan et al., 1992; Conraths and Deplazes, 2015) |

4.4. Recommendation

Table 9 lists the pathogens (parasites) such as *Ascaris*, *Ancylostoma duodenale*, *Toxocara spp.*, *Trichinella spp.*, *Entamoeba histolytica*, *Echinococcus multilocularis* and *Echinococcus granulosus* and the likely levels in urban wastewater and hospital waste; the presence of these pathogens in FYM&S is rare. It is not recommended to mix urban wastewater with FYM&S in an AD plant, hence limiting the likely presence of these parasites. Finally, this study looked to identify the top-ranked pathogens

Table 10

| Number | Pathogens | BM | Scenario A FOODIRE | Scenario B FOODEU | SUM |
|--------|-----------|----|---------------------|-------------------|-----|
| 1      | *Cryptosporidium parvum* |   | 1                   | 1                  | 3   |
| 2      | *Salmonella enterica* spp. |   | 1                   | 1                  | 1   |
| 3      | *Norovirus* |   | 1                   | 1                  | 1   |
| 4      | *Streptococcus pyogenes* |   | 1                   | 1                  | 2   |
| 5      | *Entamoeba histolytica* |   | 1                   | 1                  | 2   |
| 6      | *E. coli* enteropathogenic (EPEC) |   | 1                   | 1                  | 2   |
| 7      | *Mycobacterium* spp. |   | 1                   | 1                  | 2   |
| 8      | *Salmonella* typhi followed by *S. paratyphi* |   | 1                   | 1                  | 2   |
| 9      | *Clostridium* spp. |   | 1                   | 1                  | 2   |
| 10     | *Listeria monocytogenes* |   | 1                   | 1                  | 2   |
| 11     | *Campylobacter coli* |   | 1                   | 1                  | 2   |
| 12     | *Ascaris* spp. |   | 1                   | 1                  | 1   |
| 13     | *Giardia* lamblia, *Giardia intestinalis* |   | 1                   | 1                  | 1   |
| 14     | *Shigella* spp. |   | 1                   | 1                  | 1   |
| 15     | *Enterobacter* spp. |   | 1                   | 1                  | 1   |
| 16     | *Toxoplasma gondii* |   | 1                   | 1                  | 1   |
| 17     | *Brucella* spp. |   | 1                   | 1                  | 1   |
| 18     | *Coxiella burnetti* |   | 1                   | 1                  | 1   |
| 19     | *Echinococcus* spp. |   | 1                   | 1                  | 1   |
| 20     | *Yersinia enterocolitica* |   | 1                   | 1                  | 1   |
| 21     | *Campylobacter jejuni* |   | 1                   | 1                  | 1   |
| 22     | *Vibrio* spp. |   | 1                   | 1                  | 1   |
| 23     | *Hepatitis* A-virus |   | 1                   | 1                  | 1   |
| 24     | *E. coli* O157:H7 |   | 1                   | 1                  | 1   |
| 25     | *E. coli* invasive & toxigenic |   | 1                   | 1                  | 1   |
| 26     | *Streptococcus pneumoniae* |   | 1                   | 1                  | 1   |
| 27     | *Rotavirus* |   | 1                   | 1                  | 1   |

Note: Highlighted pathogens are present in municipal wastewater only (Table 9) and therefore not considered.
Ireland, the co-digestion of urban wastewater and FYM&S is unlikely (Singh et al., 2010). Hence, Entamoeba histolytica may be excluded at this final stage of the hazard identification for Ireland.

4.5. Limitations and future work

i. Plant pathogens were not considered.

ii. Detailed thermal inactivation data (Tref, Dvalue and Zvalue) of animal pathogens (which cause illness to animals only, not human) is unavailable; hence, comparison with indicators was the only possible way to investigate them.

iii. The model can be improved in the future when the mortality rate for all 91 pathogens will be available and S3 score could be based on the mortality rate only.

5. Conclusion

This study developed a simple risk ranking methodology based upon inactivation of pathogens during AD, hazard pathway routes and human mortality rates. Cryptosporidium parvum, Salmonella spp., norovirus, Strep tococcus pyogenes, E. coli enteropathogenic (EPEC), Mycobacterium spp., Salmonella typhi (followed by S. paratyphi), Clostridium spp., Listeria monocytogenes and Campylobacter coli were found to be the most relevant (top 10) pathogens in relation to potential risk from spreading anaerobic digestate on agricultural land, specifically in Ireland. The score corresponding to the mortality rate (S3) was the most sensitive parameter (rank coefficient 0.49) to the final score S; followed by thermal inactivation score S1 (0.25) and potential contamination pathways S2 (0.16). A complete risk assessment of top-ranked pathogens can unify the data collected from the laboratory and field experiments into comprehensible statistics and predict potential risk which could help relevant agencies and government authorities to take the necessary steps to identify the most sensitive pathways or processes responsible for the overall risk and thus, act to minimise potential risk.

CRediT authorship contribution statement

Rajat Nag: Conceptualization, Methodology, Software, Data curation, Visualization, Investigation, Writing - original draft.
Paul Whyte: Writing - review & editing.
Bryan K. Markey: Writing - review & editing.
Vincent O’Flaherty: Writing - review & editing.
Declan Bolton: Writing - review & editing.
Owen Fenton: Writing - review & editing.
Karl Richards: Writing - review & editing.
Enda Cummins: Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.136297.

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