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

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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 monocytogenes* and *Campylobacter coli* as the highest-ranking pathogens of human health concern resulting from AD digestate in Ireland. This tool prioritises potentially harmful pathogens which can emerge from AD digestate and highlights where regulation and intervention may be required.

Keywords

Hazard identification, semi-quantitative screening, anaerobic digestion, pasteurisation, risk assessment

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). Hutchison *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 (Szogi *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 (Abbasi *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; Abbasi *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; Abbasi *et al.* 2012; Manyi-Loh *et al.* 2013).

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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 hour 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, measles 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 measles 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

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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, agriculture 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, 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.

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2.1.1. Initial hazard selection

Using the scientific literature (Carrington 2001; Jones and Martin 2003; Lepeuple *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 $\log_{10}$ (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.

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For example, enterohemorrhagic *E. coli* O157: H7 can be inactivated at 55 °C for 40 minutes; Equation 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 mins (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 minutes or 45 °C for 24 h (Table S2); therefore, 55 °C for 40 minutes was adopted for the inactivation reference as it is closer to the mean temperature (62.4 °C) of comparable scenarios (Table 1).

$$New\ Dvalue\ (min)\ = Dref \div 10^{([Tnew-Tref)]/Zvalue)} \ldots \text{Equation 1}$$

Where,

Tref (°C) = reference temperature from the literature at which the time-temperature inactivation tests were done; for enterohemorrhagic *E.coli* O157: H7 example, say 55 °C (from Table S2).

Dref (min) = duration of heating at Tref for the experiment considering complete inactivation of the pathogen; for the above example, say 40 min (from Table S2).

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

Tnew (°C) = 37 °C (mesophilic condition)

New Dvalue (min) (for mesophilic condition) = 40 ÷ $10^{((37 - 55) / 9.15)}$ = 3709 min (2.57 days). This New Dvalue is used to score (S1) pathogens (Equation 2).

Similarly, new Dvalue (min) 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, 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 (S1) 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, S1 is set to 0.001

otherwise,

$$S1 = \frac{New\ Dvalue - process\ duration}{New\ Dvalue} \text{ …Equation 2}$$

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 S2 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 S3 represents the mortality rate from 0.1 to 1 (Fig. 2 and 3) where 0.1 stands for 10 % and 1 corresponds to 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 more than 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 less than

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1 per 100,000 population; the value between 1 to 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 × S) matrix. The final score S of the screening process was based on the multiplication of three individual scores S1, S2 and S3 (Equation 3). The scores were multiplied so the absence of any one score will result in the elimination of risk.

$$S = S1 \times S2 \times S3 \ldots \text{Equation 3}$$

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 source), survival conditions (aerobic/ anaerobic/ facultative/ obligate), classification types (Gram-positive/ negative), spore/ egg forming 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.

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.

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2.2.1. Scenario 1: Model considering only foodborne illness in Ireland (Scenario A $_{FOODIRE}$)

The methodology for Scenario A $_{FOODIRE}$ 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 (notification rates) in Ireland (Table 5) was collected for each target pathogens from the EFSA reports (European Food Safety Authority 2009, 2010, 2011, 2012, 2013, 2014, 2015a, 2015b, 2016, 2017). Data for *Cryptosporidium* was collected from the Health Protection Surveillance Centre (HPSC) (2018). An appropriate relative score $S3_{IRE}$ (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). Next, the final score (S) was calculated as $S1 \times S2 \times S3_{IRE}$ similar to Equation 3.

2.2.2. Scenario 2: Model considering only foodborne illness in Europe (Scenario B $_{FOODEU}$)

Comparing to the Scenario A $_{FOODIRE}$ 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 $S3_{EU}$ (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 Equation 3, the final score (S) was calculated as $S1 \times S2 \times S3_{EU}$.

2.2.3. Scenario 3: Model considering raw manure and slurry application without heat treatment and AD (Scenario C $_{RAWFYM\&S}$)

In this scenario (Scenario C $_{RAWFYM\&S}$), 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 or anaerobic) of the pathogens, Gram +/ - ve, zoonotic nature, spore/ cyst/ egg forming ability, incubation period, growth/ re-growth ability, and infectious dose (organisms) which helped to select indicators (Table 2) for this study. The physical inactivation data (time-temperature) and the 'Z' values were collected from available literature and summarised in Table S2. Applying Equation 1, new D values were calculated, and is presented in Table S4 for new temperature (Tnew) conditions (37 °C, 55 °C, 60 °C, 70 °C, and 90 °C). An appropriate score (S3) was given according to Equation 2 and the results are listed in Table S6 under the S1 column. Next, the second score S2 was evaluated accumulating the individual scores for different pathways and are described in Table S5. After this process, the third score (S3, Table S6) which is based on the mortality rate was applied to the pathogens comparing Table S1 and S2.

3.2. The baseline model (BM)

The three scores (S1, S2 and S3) were multiplied to get the final score (S) as presented in Table S6 and Fig. 4. The maximum value was obtained for *Cryptosporidium parvum* (0.9). The highest-ranked 14 pathogens are plotted on a bar chart (Fig. 5) according to their order from high to low as *Cryptosporidium parvum*, *Streptococcus pyogenes*, *Entamoeba histolytica*, *Salmonella enterica* spp., *Ascaris* spp., enteropathogenic *E. coli* (EPEC), *Mycobacterium* spp., *Salmonella typhi* (followed by *S. paratyphi*), *Giardia lamblia* and *Giardia intestinalis*, *Shigella* spp., norovirus, *Enterobacter* spp., *Clostridium* spp. and *Listeria monocytogenes*. Fig. 6 indicated the final score (S) was less than 0.1 and for 48 pathogens whereas, only 11 pathogens scored more than 0.4. A comparison between the top-ranked pathogens (BM scenario) and the indicator pathogens is presented in Table 7.

3.3. Scenarios

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The food scenarios (Scenario A $_{FOODIRE}$ and Scenario B $_{FOODEU}$) identified the top-ranked pathogens which are presented in the bar charts Fig. 7a and 7b, respectively. These pathogens are *Cryptosporidium parvum*, *Salmonella enterica* spp., *Mycobacterium* spp., *E. coli* enteropathogenic (EPEC), *Toxoplasma gondii*, *Listeria monocytogenes*, norovirus, *Clostridium* spp., *Coxiella burnetti*, *Brucella* spp., *Yersinia enterocolitica*, *Echinococcus* spp., *Trichinella* spp., *Campylobacter coli*, *Vibrio* spp. and hepatitis A-virus. The top 12 pathogens were ranked for Scenario C $_{RAWFYM\&S}$ (Fig. 7c) and these are, *Cryptosporidium parvum*, *Campylobacter coli*, *Campylobacter jejuni*, *E-coli* enterohamorrhagic (verotoxin) O157:H7, *E. coli* invasive & toxigenic, *Salmonella enterica* spp., norovirus, *Salmonella typhi*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Entamoeba histolytica* and rotavirus.

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 $_{FOODEU}$ (Fig. 7b), and Scenario C $_{RAWFYM\&S}$ (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 $_{FOODEU}$ (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 $_{RAWFYM\&S}$), 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,

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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). Figure 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 (Fig. 4). 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 be used to assess

the pathogens of an animal health concern as a comparison between these pathogen 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.

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 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 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, *Streptococcus 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.

Author contributions

Rajat Nag: conceptualization, methodology, software, data curation, visualization, investigation, writing - original draft preparation. Enda Cummins: conceptualization, supervision, writing - reviewing and editing. Paul Whyte, Bryan K Markey, Vincent O’Flaherty, Declan Bolton, Owen Fenton, Karl Richards: writing - reviewing and editing.

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Author contributions

Rajat Nag: conceptualization, methodology, software, data curation, visualization, investigation, writing - original draft preparation. Enda Cummins: conceptualization, supervision, writing - reviewing and editing. Paul Whyte, Bryan K Markey, Vincent O'Flaherty, Declan Bolton, Owen Fenton, Karl Richards: writing - reviewing and editing.

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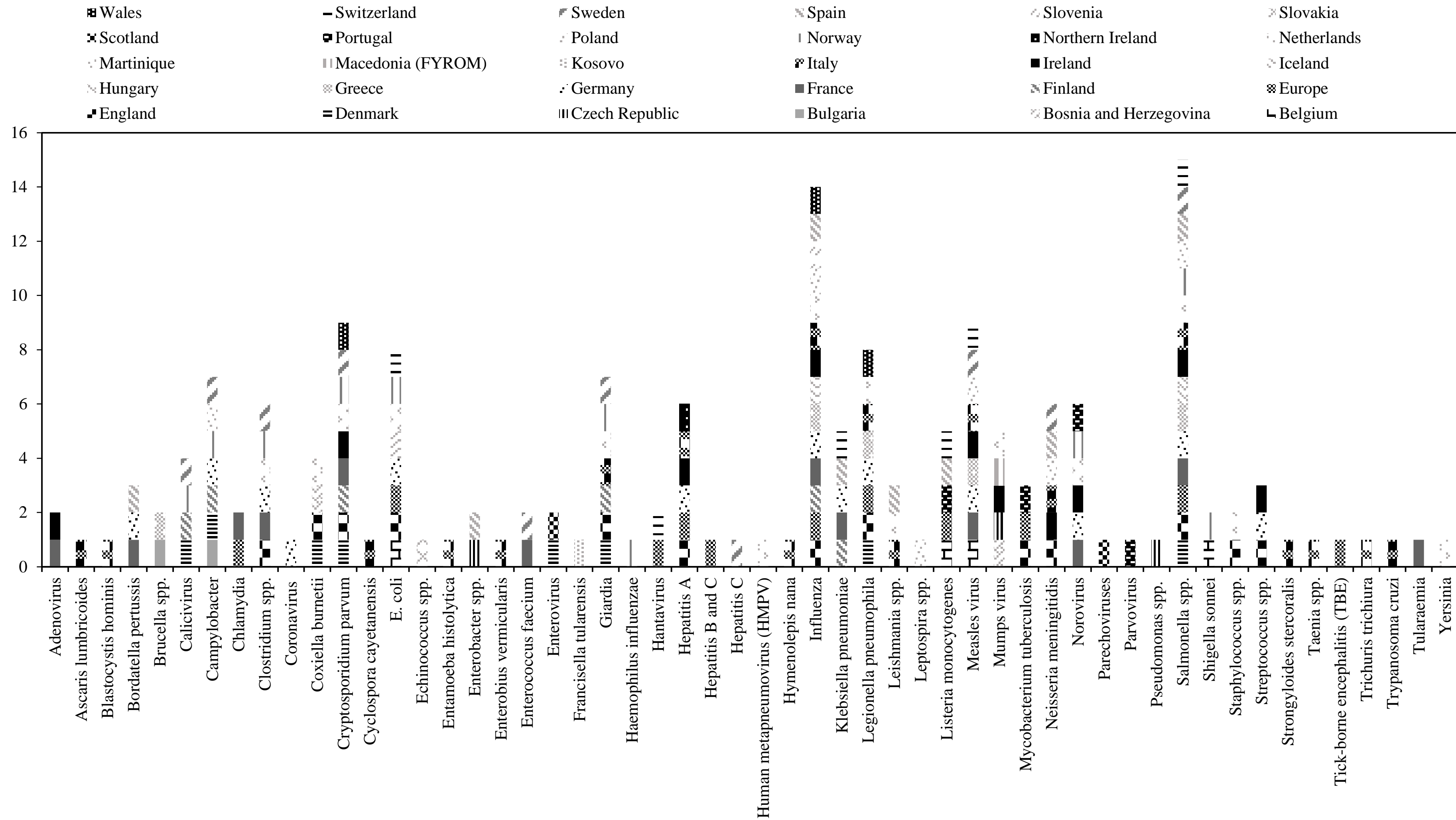

Fig. 1. Observed human disease outbreaks in Europe (last 20 years)

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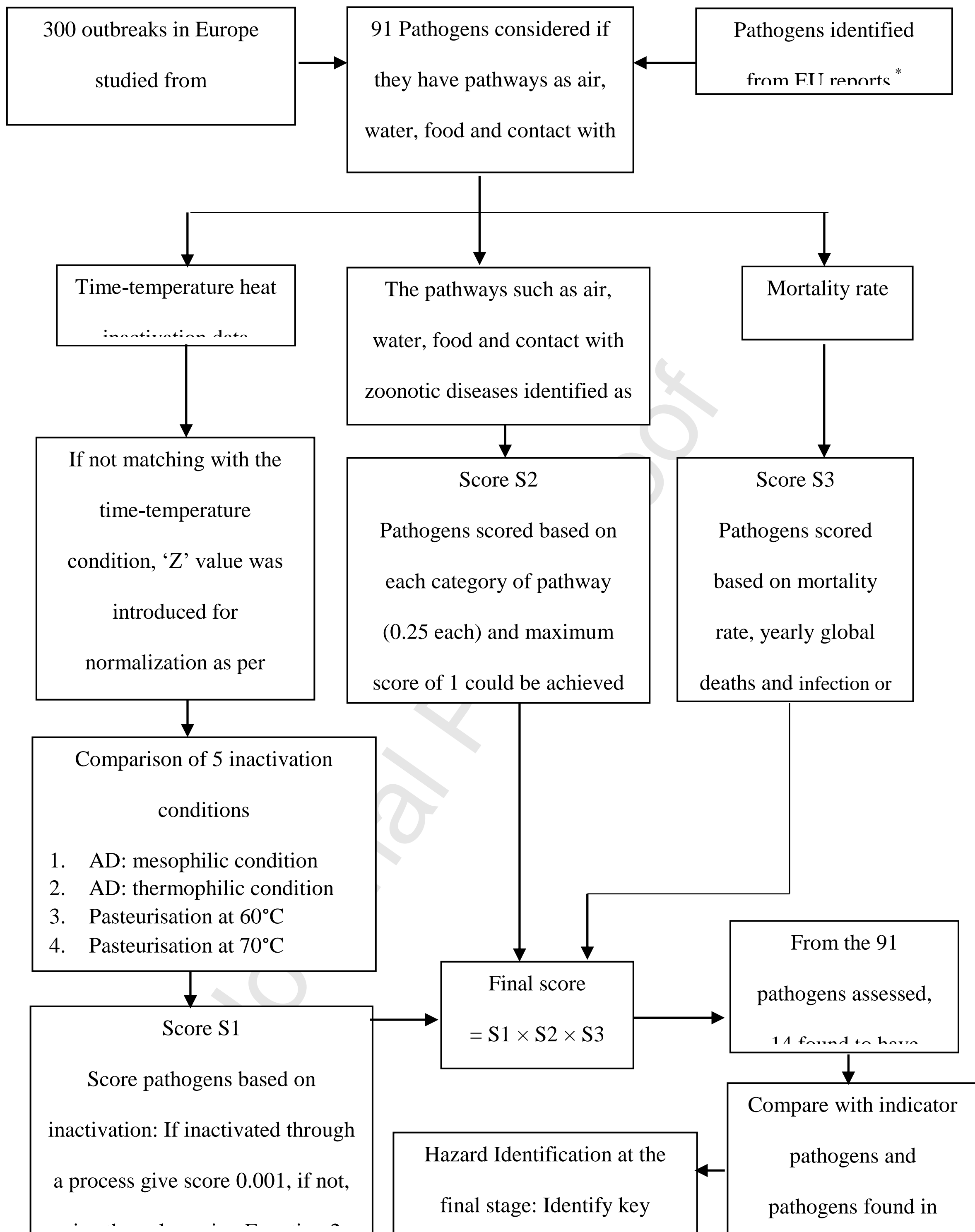

Fig. 2. Flow diagram of the screening method

* Reference (Carrington 2001; Jones and Martin 2003; Lepeuple *et al.* 2004; WHO 2008; Bøtner and Belsham 2012; Longhurst *et al.* 2013; Torgerson *et al.* 2015)

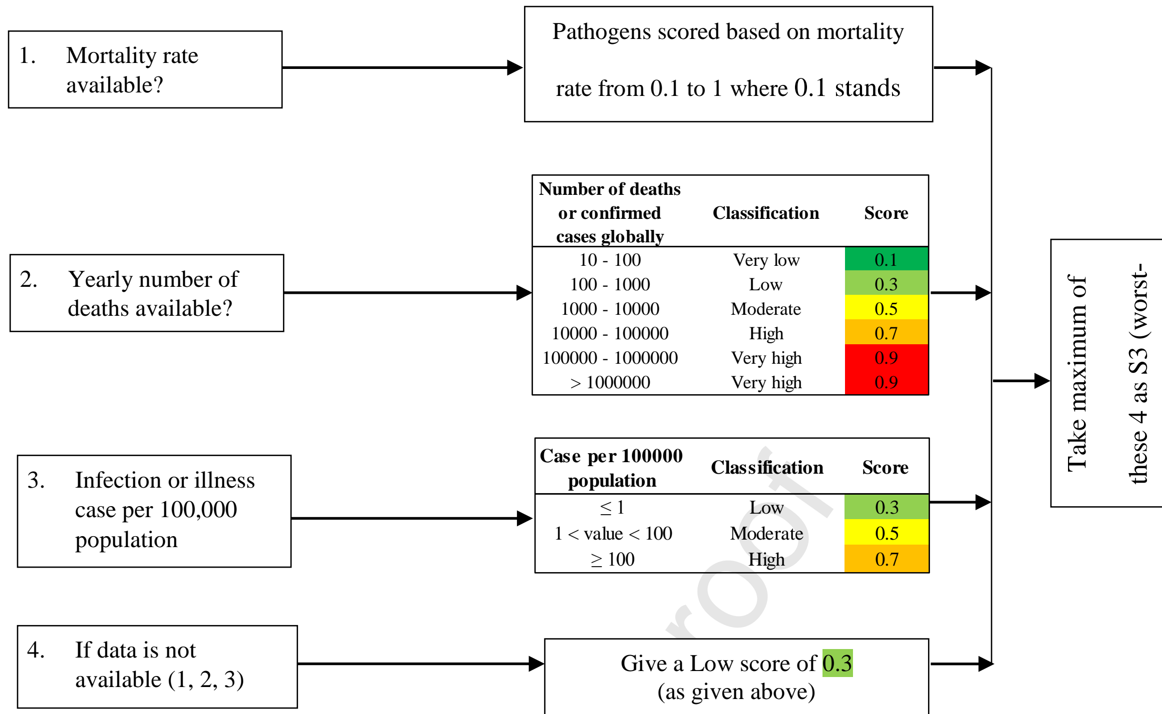

Fig. 3. Adopted strategy for S3 scoring

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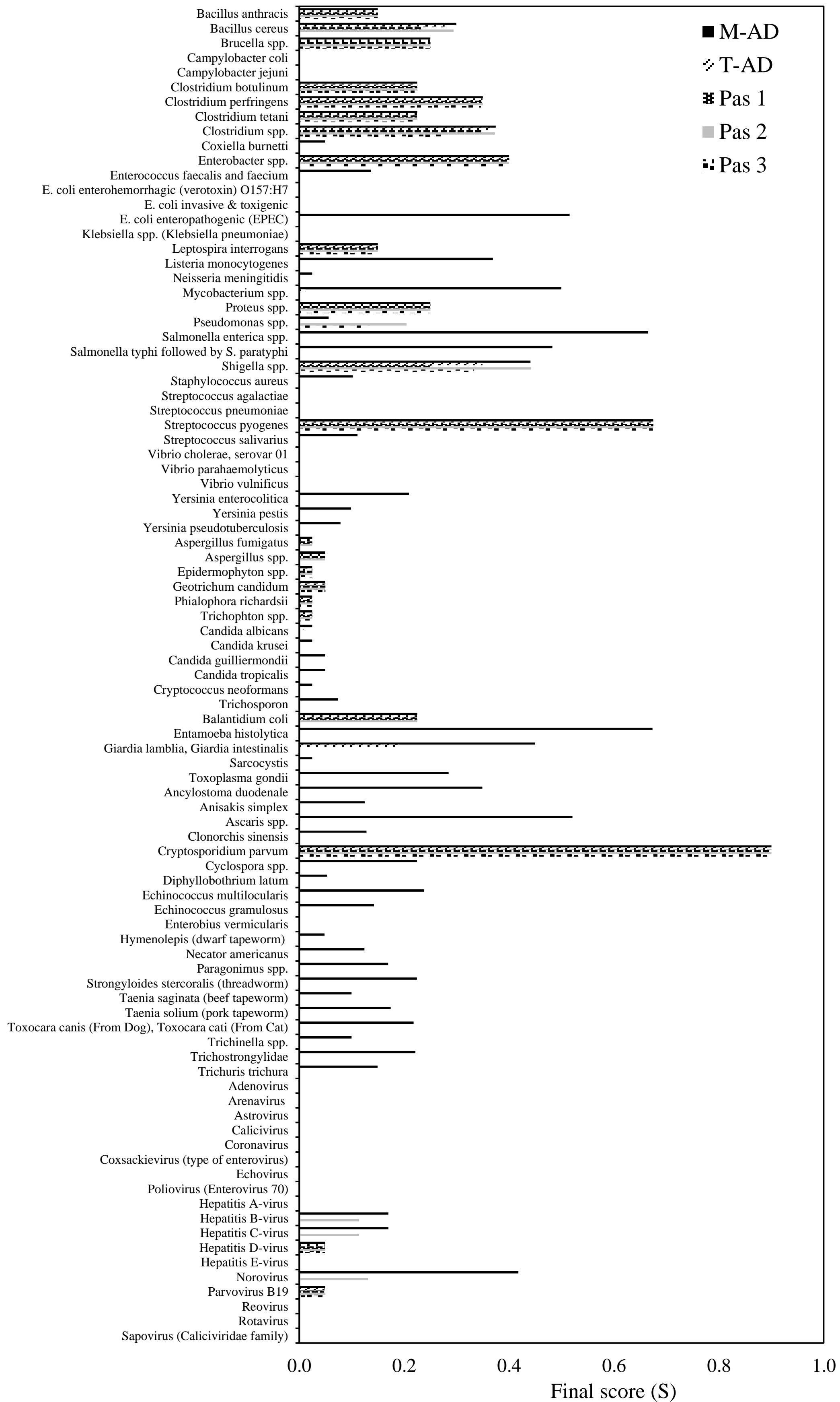

Bacillus anthracis
Bacillus cereus
Brucella spp.
Campylobacter coli
Campylobacter jejuni
Clostridium botulinum
Clostridium perfringens
Clostridium tetani
Clostridium spp.
Coxiella burnetti
Enterobacter spp.
Enterococcus faecalis and faecium
E. coli enterohemorrhagic (verotoxin) O157:H7
E. coli invasive & toxigenic
E. coli enteropathogenic (EPEC)
Klebsiella spp. (Klebsiella pneumoniae)
Leptospira interrogans
Listeria monocytogenes
Neisseria meningitidis
Mycobacterium spp.
Proteus spp.
Pseudomonas spp.
Salmonella enterica spp.
Salmonella typhi followed by S. paratyphi
Shigella spp.
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarius
Vibrio cholerae, serovar 01
Vibrio parahaemolyticus
Vibrio vulnificus
Yersinia enterocolitica
Yersinia pestis
Yersinia pseudotuberculosis
Aspergillus fumigatus
Aspergillus spp.
Epidermophyton spp.
Geotrichum candidum
Phialophora richardsii
Trichophton spp.
Candida albicans
Candida krusei
Candida guilliermondii
Candida tropicalis
Cryptococcus neoformans
Trichosporon
Balantidium coli
Entamoeba histolytica
Giardia lamblia, Giardia intestinalis
Sarcocystis
Toxoplasma gondii
Ancylostoma duodenale
Anisakis simplex
Ascaris spp.
Clonorchis sinensis
Cryptosporidium parvum
Cyclospora spp.
Diphyllobothrium latum
Echinococcus multilocularis
Echinococcus gramulosus
Enterobius vermicularis
Hymenolepis (dwarf tapeworm)
Necator americanus
Paragonimus spp.
Strongyloides stercoralis (threadworm)
Taenia saginata (beef tapeworm)
Taenia solium (pork tapeworm)
Toxocara canis (From Dog), Toxocara cati (From Cat)
Trichinella spp.
Trichostrongylidae
Trichuris trichura
Adenovirus
Arenavirus
Astrovirus
Calicivirus
Coronavirus
Coxsackievirus (type of enterovirus)
Echovirus
Poliovirus (Enterovirus 70)
Hepatitis A-virus
Hepatitis B-virus
Hepatitis C-virus
Hepatitis D-virus
Hepatitis E-virus
Norovirus
Parvovirus B19
Reovirus
Rotavirus
Sapovirus (Caliciviridae family)
M-AD
T-AD
Pas 1
Pas 2
Pas 3
0.0
0.2
0.4
0.6
0.8
1.0
Final score (S)

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Fig. 4. The result of the screening model with five different conditions (**BM**)

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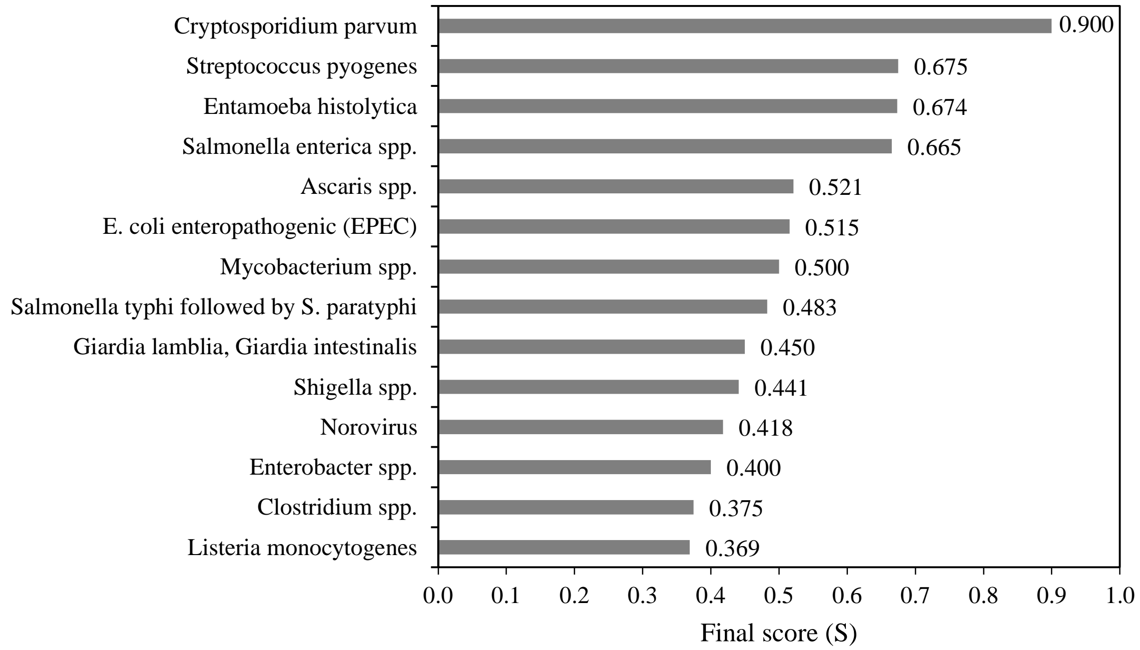

Fig. 5. Ranking of top 14 pathogens based on qualitative screening process (**BM**)

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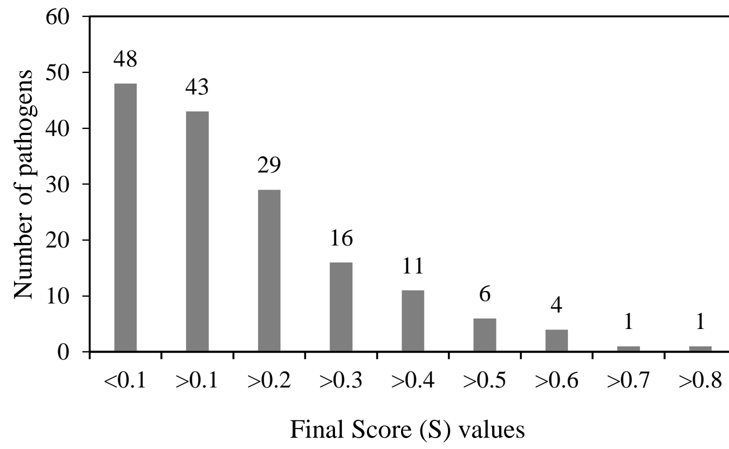

Fig. 6. Bin distribution of the final score (S)

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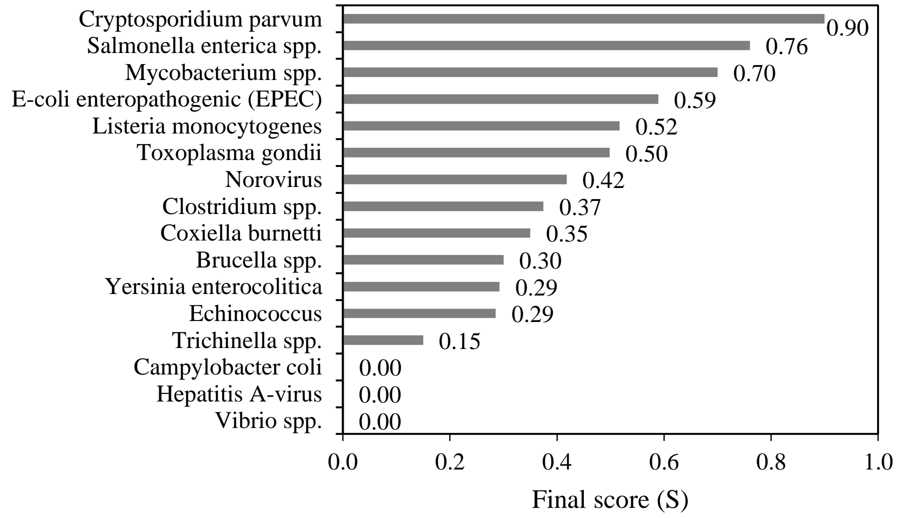
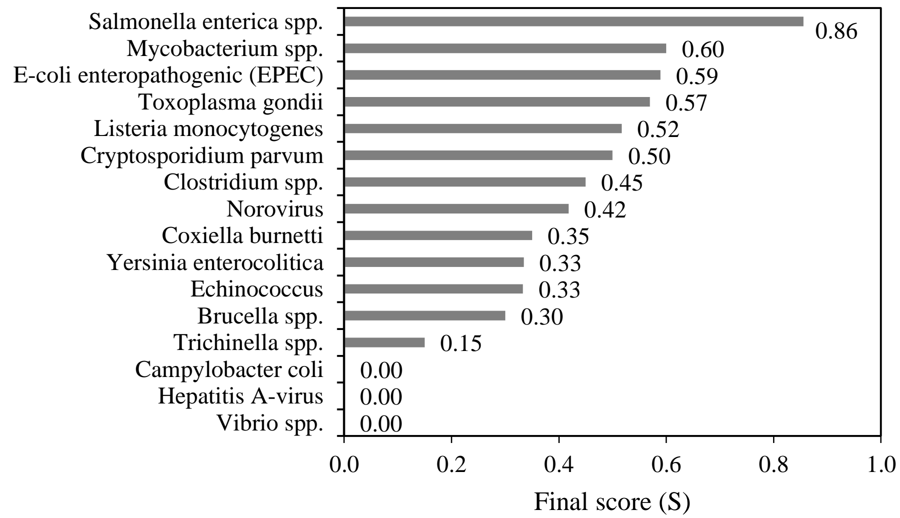
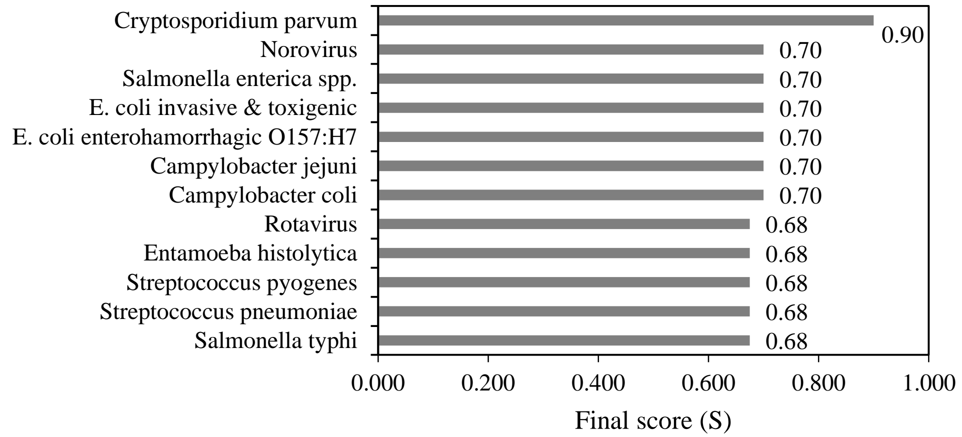


a) **Scenario A** $_{\textbf{FOODIRE}}$



b) **Scenario B** $_{\textbf{FOODEU}}$



c) **Scenario C** $_{\mathbf{RAWFYM\&S}}$

Fig. 7. Ranking of top pathogens in different scenarios

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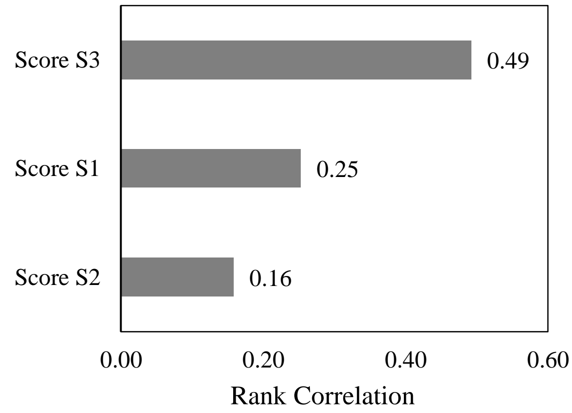

Fig. 8. The correlation coefficient (Spearman rank) of three different scores S1, S2 and S3 for the top 14 pathogens (**BM**)

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Table 10	Final comparison checklist and selection of top-ranked pathogens

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Table 1: Time-temperature conditions studied

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

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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-llivina *et al.* 2003)
Enterococcus faecalis	Gram + ve, non-spore forming bacteria	(McFeters *et al.* 1974; Mocé-llivina *et al.* 2003; Sahlström 2003; Anderson *et al.* 2005; Sidhu and Toze 2009)
Clostridium spp.	Gram + ve, spore-forming bacteria	(Payment and Franco 1993; Ferguson *et al.* 1996; Fewtrell and Bartram 2001)
Mycobacterium spp.	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)

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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, pleuropneumonia and parasitic bronchitis)	*Mycobacterium*, Bovine respiratory syncytial virus (RSV), *Trueperella pyogenes*, *Mannheimia haemolytica*, *Dictyocaulus* spp., *Mycoplasma bovis*, *Pasteurella multocida*, bovine herpesvirus, *Histophilus somni*	17
Systemic infection	*Escherichia coli*	5
Clostridial infection	*Clostridium novyi*, *Cl. Chauvoei*, *Cl. Sordellii*, *Cl. perfringens*, *Cl. septicum*, *Cl. perfringens*, *Cl. Botulinum*	4
Cardiac infection	*Trueperella pyogenes*	9.5
Liver disease	*Listeria monocytogenes*, Liver fluke	3.5
Bovine abortion	*Trueperella pyogenes*, *Salmonella Dublin*, *Bacillus licheniformis*, *Listeria moncytogenes*, *Aspergillus* spp.	7.1, 4.8, 4.1, 2.9, 0.6
Bovine mastitis	*E. coli, Staphylococcus aureus, Streptococcus uberis*	8, 26.8, 12
Sheep		
Parasitic disease	*Teladorsagia (Ostertagia) circumcincta*, *Haemonchus contortus*, *Trichostrongylus spp*, *Nematodirus battus*	13
Respiratory infections	*Mannheimia haemolytica*, Less commonly (*Pasteurella multocida*, *Trueperella pyogenes*, *Bibersteinia trehalosi* and *Mycoplasma ovipneumonae*)	12
Septicaemia	*Bibersteinia trehalosi*	15
Clostridial and Kidney disease	*Clostridium perfringens*, *Clostridium difficile*	7
Enteric disease	rotavirus and coronavirus	7
Ovine abortion	*Toxoplasma gondii*, *Chlamydophila abortus*, *E. coli*, *Salmonella*	40.2, 26.1, 16.5, 0.8,

	Dublin, *Trueperella pyogenes*, *Listeria* spp., *Streptococcus* spp.	4.4, 4.0, 2.0
	Pig	
Pneumonia	*Pasteurella multocida*, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropeumoniae*, *Trueperella pyogenes*, Swine influenza virus	29
Colibacillosis and Enteric infection	*E. coli*, *Salmonella*, *Clostridium perfringens*, *Clostridium difficile*	22
Septicaemia	*Klebsiella pneumoniae*, *Streptococcus suis*, *Listeria monocytogenes*, *E. coli*	12
Nervous disease	*Streptococcus suis*	5
	Poultry	
Septicaemia	*Escherichia coli*, *Erysipelothrix rhusiopathiae*	26
Digestive	*Erysipelothrix rhusiopathiae*, *Brachyspira* spp., adenovirus	6.5
Musculoskeletal	NA	8
Respiratory	Adenovirus	9
Parasitic disease	*Dermanyssus gallinae*	15

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Table 4: Scenarios considered

Number	Name	Description	Difference from BM	Final score S
1	Scenario A $_{FOODIRE}$	Model considering only foodborne illness in Ireland	S3 based on foodborne illness in Ireland ($S3_{IRE}$)	$S1 \times S2 \times S3_{IRE}$
2	Scenario B $_{FOODEU}$	Model considering only foodborne illness in the Europe	S3 based on foodborne illness in the Europe ($S3_{EU}$)	$S1 \times S2 \times S3_{EU}$
3	Scenario C $_{RAWFYM\&S}$	Model considering raw FYM&S application without heat treatment and AD	No S1, only S2 and S3	$S2 \times S3$

Note: The final score S for baseline model (BM) was calculated as $S1 \times S2 \times S3$ (Equation 3).

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Table 5: Pathogens considered for Scenario A $_{FOODIRE}$

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]
		2016	2015	2014	2013	2012	2011	2010	2009	2008	2007	2016	2015	2014	2013	2012	2011	2010	2009	2008	2007		
1	*Campylobacter* spp.	2511	2,453	2,593	2,288	2391	2433	1660	1810	1752	1885	53.1	53	56.3	49.8	52.17	54.3	37.15	40.67	39.8	43.7	47.999	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	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	0	5		9	17			0.13	0.09	0	0	0.11		0.2	0.4			0.132	0.7
7	*Echinococcus* spp.	2	0	0	1	1	0	1	1	2	0	0.04	0	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	7	0.04	0	0.07	0.02	0.04	0.02	0.02	0	< 0.1	0.2	0.045	0.6
9	*Trichinella* spp.	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	< 0.1	0.09	0.6
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	0.16	0.25	0.11	< 0.1	0.122	0.7
11	*Toxoplasma gondii*	0	1	0	1	1		1	37			0	1.5	0	1.5	1.4		1.36	0.83			0.941	0.7
12	*Vibrio* spp.																					0.001	0.5
13	*Clostridium* spp.																					0.001	0.5
14	Norovirus							50	28									1.1	0.616			0.858	0.7
15	*Hepatitis A*																					0.001	0.5
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	10.755	0.9

Note:
1. Scale for selecting score $S3_{IRE}$ based on the total number of confirmed cases/100,000 population (notification rates)
2. Iceland, Norway, Switzerland are excluded; no special agreement for data
3. Black cells represent unavailability of data in the report
4. ♦ 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

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Table 6: Pathogens considered for Scenario B $_{FOODEU}$

Number	Pathogens	Number of confirmed human cases in the EU										Total number of confirmed cases/100,000 population (notification rates) *										Avg. value	Score $S3_{EU}$[1]
		2016	**2015**	**2014**	**2013**	**2012**	**2011**	**2010**	**2009**	**2008**	**2007**	**2016**	**2015**	**2014**	**2013**	**2012**	**2011**	**2010**	**2009**	**2008**	**2007**		
1	*Campylobacter* spp.	246307	232134	236818	214710	214300	220209	215397	198725	190579	200980	66.3	62.9	66.5	61.4	61.7	50.28	48.56	45.57	40.7	45.2	54.911	0.9
2	*Salmonella* spp.	94530	94597	92012	87753	94278	95548	101037	110181	134580	153852	20.4	20.9	20.7	20.3	21.9	20.7	21.5	24	26.4	31.1	22.79	0.9
3	*Yersinia* spp.	6861	6928	6435	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	1.887	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	1.356	0.8
5	*Listeria monocytogenes*	2536	2206	2242	1883	1720	1476	1601	1654	1425	1581	0.47	0.43	0.46	0.39	0.36	0.32	0.35	0.36	0.3	0.3	0.374	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	0.189	0.7
8	*Brucella* spp.	516	437	462	498	503	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	0.092	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	0.03	0.02	0.02	< 0.1	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			4.121	0.8
12	*Vibrio* spp.	76	29						17			< 0.01	< 0.01									0.009	0.5
13	*Clostridium* spp.	49	60	1727	2009	1729	1050	795	1704	857		0.01	0.01	0.04	0.06	0.03	0.03	0.02	0.03	0.03		0.028	0.6
14	Norovirus	11993	13536	3580	2023	13987	2529	6533	2670	3617		0.08	0.06		0.23							0.123	0.7
15	*Hepatitis A*	155	78	48	1444	116	7	13	2	104		< 0.01	< 0.01	< 0.01								0.009	0.5
16	*Cryptosporidium*	62	120	24	65	11	20000	12700		87		< 0.01	< 0.01	< 0.01								0.009	0.5

Note:
1. Scale for selecting score $S3_{EU}$ based on the total number of confirmed cases/100,000 population (notification rates)
2. Iceland, Norway, Switzerland are excluded; no special agreement for data
3. Black cells represent unavailability of data in the report

* Number of confirmed cases/100,000 population range		Score $S3_{EU}$
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

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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	*Cryptosporidium*
4	*Salmonella enterica* spp.	Gram - ve, facultative anaerobe, non-spore forming, coliform bacteria	Itself *Salmonella senftenberg* (more heat resistant)
5	*Ascaris* spp.	Parasites: helminths	*Cryptosporidium*
6	*E. coli enteropathogenic (EPEC)*	Gram -ve, facultative anaerobe, non-spore forming coliform bacteria	Itself
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 *Salmonella senftenberg* (more heat resistant)
9	*Giardia lamblia, Giardia intestinalis*	Parasites: Protozoa	*Cryptosporidium*
10	*Shigella spp.*	Gram - ve, facultative anaerobe, non-spore forming, coliform bacteria	*E. coli, Salmonella senftenberg*
11	Norovirus (surrogated by FCV)	Virus	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*

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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)[1]	African Swine Fever (ASF)	Virus					Feline calicivirus (FCV)
3	*Babesia* spp.	Babesiosis/ tick-borne disease	protozoan parasite	✓				Cryptosporidium parvum
4	*Bibersteinia trehalosi*	Pneumonia	Gram-negative, facultative anaerobic bacteria	✓	✓			Escherichia coli/ Salmonella enterica spp.
5	Bluetongue virus[1]	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	*Chlamydophila abortus*	Abortion 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	*Coccidian 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	*Echinostomida* spp.	Paramphistomosis	Parasites: helminths		✓			Ascaris/ Cryptosporidium parvum
15	*Eimeria* spp.	Coccidiosis	protozoan parasites	✓	✓			Cryptosporidium parvum
16	*Erysipelothrix rhusiopathiae*[2]	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[1]	Newcastle Disease	Virus					Feline calicivirus (FCV)
24	*Pasteurella* spp.	Septicaemia	Gram-negative, facultative anaerobic bacteria	✓	✓	✓		Escherichia coli/ Salmonella enterica spp.
25	Retrovirus[1]	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

Note:

1. 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
2. Zoonotic

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Table 9: Likely levels and sources of parasites can be found in urban wastewater and hospital waste

Pathogen name	Likely levels	Unit	Source	Reference
Ascaris	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	
Ancylostoma duodenale	100-150	eggs g^{-1}	Affected human stool	(Anderson and Schad 1985)
	Mean intensity of infection was 250.1 ± 64.4		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 × 10^2 to 5.0 × 10^2	cysts l^{-1}	Wastewater treatment plant influent	(Sabbahi *et al.* 2018)
	39 - 308	cysts g^{-1}	Faecal sample collected from infected patients in hospitals	(Voupawoe 2016)
Echinococcus multilocularis 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)

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Table 10: Final comparison checklist and selection of top-ranked pathogens

Number	**Pathogens**	**BM**	**Scenario A $_{FOODIRE}$ or Scenario B $_{FOODEU}$**	**Scenario C $_{RAWFYM\&S}$**	**SUM**
1	*Cryptosporidium parvum*	1	1	1	3
2	*Salmonella enterica spp.*	1	1	1	3
3	Norovirus	1	1	1	3
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
13	*Giardia lamblia, Giardia intestinalis*	1			1
14	*Shigella* spp.	1			1
15	*Enterobacter* spp.	1			1
16	*Toxoplasma gondi*		1		1
17	*Brucella* spp.		1		1
18	*Coxiella burnetti*		1		1
19	*Echinococcus* spp.		1		1
20	*Yersinia enterocolitica*		1		1
21	*Campylobacter jejuni*			1	1
22	*Vibrio* spp.			1	1
23	Hepatitis A-virus			1	1
24	*E-coli* O157:H7			1	1
25	*E-coli* invasive & toxigenic			1	1
26	*Streptococcus pneumoniae*			1	1
27	Rotavirus			1	1

Note: Highlighted pathogens are present in municipal wastewater only (Table 13) and therefore not considered.

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- 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.

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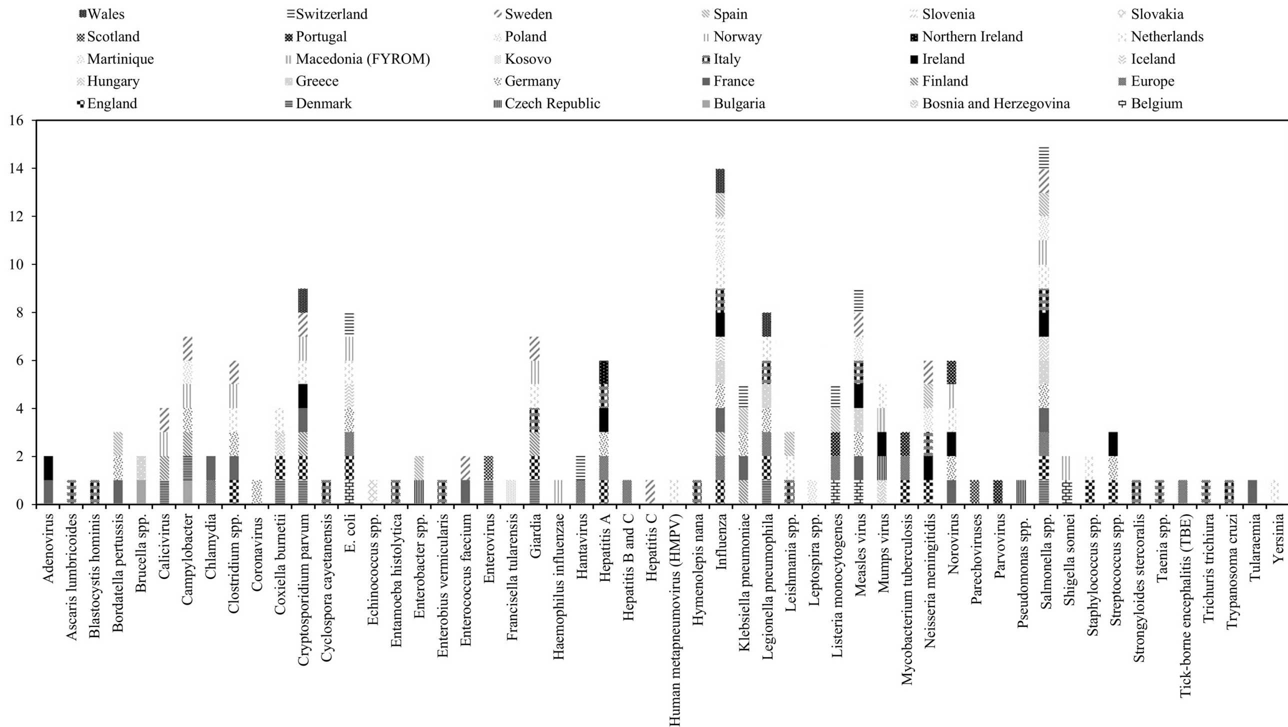


Figure 1

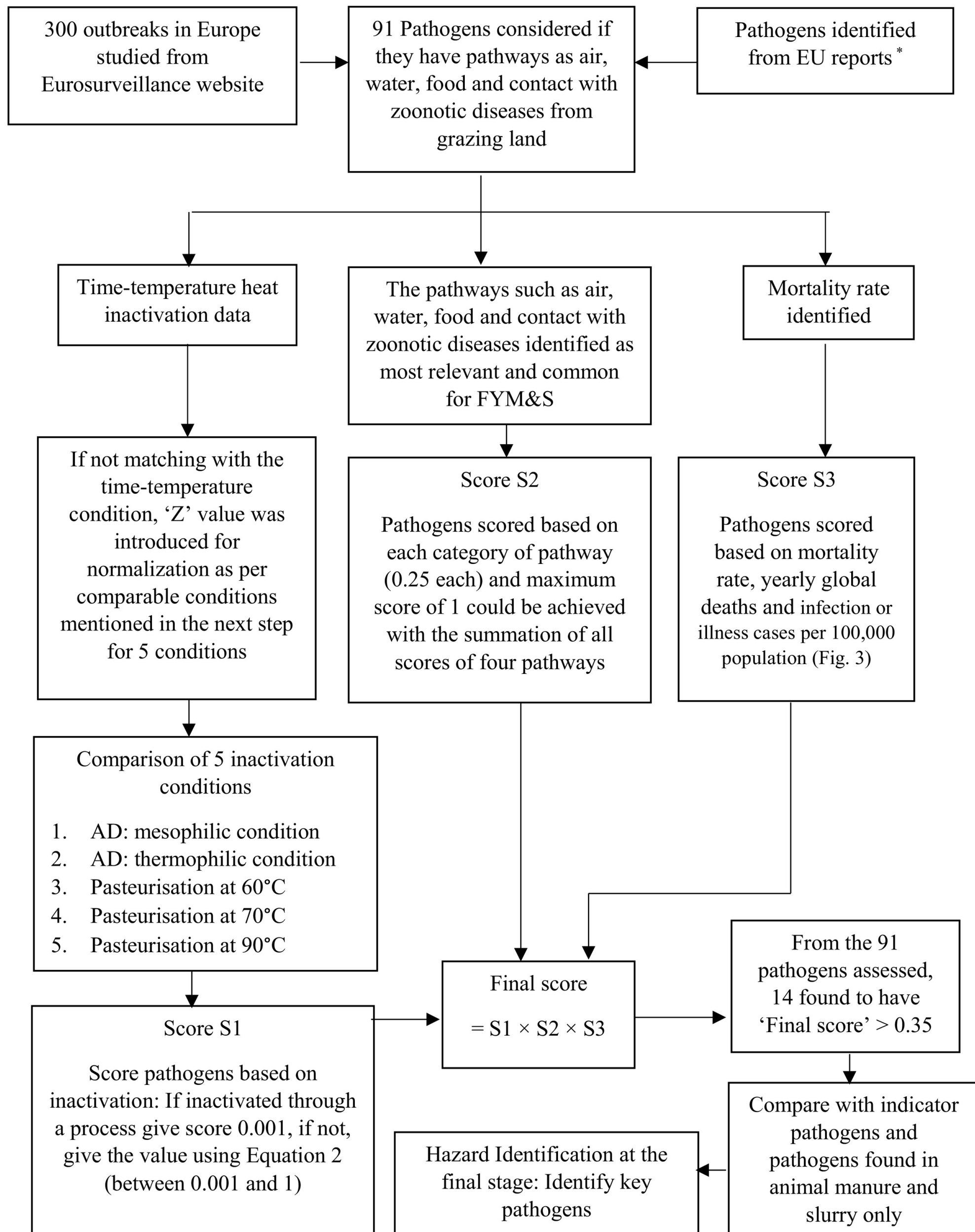


Figure 2

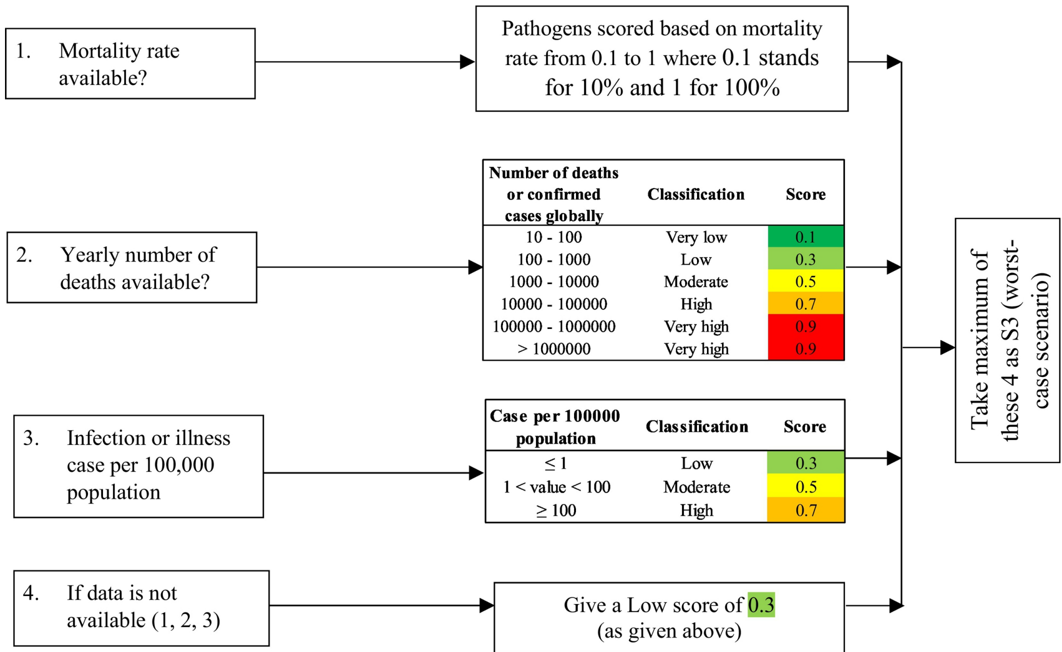


Figure 3

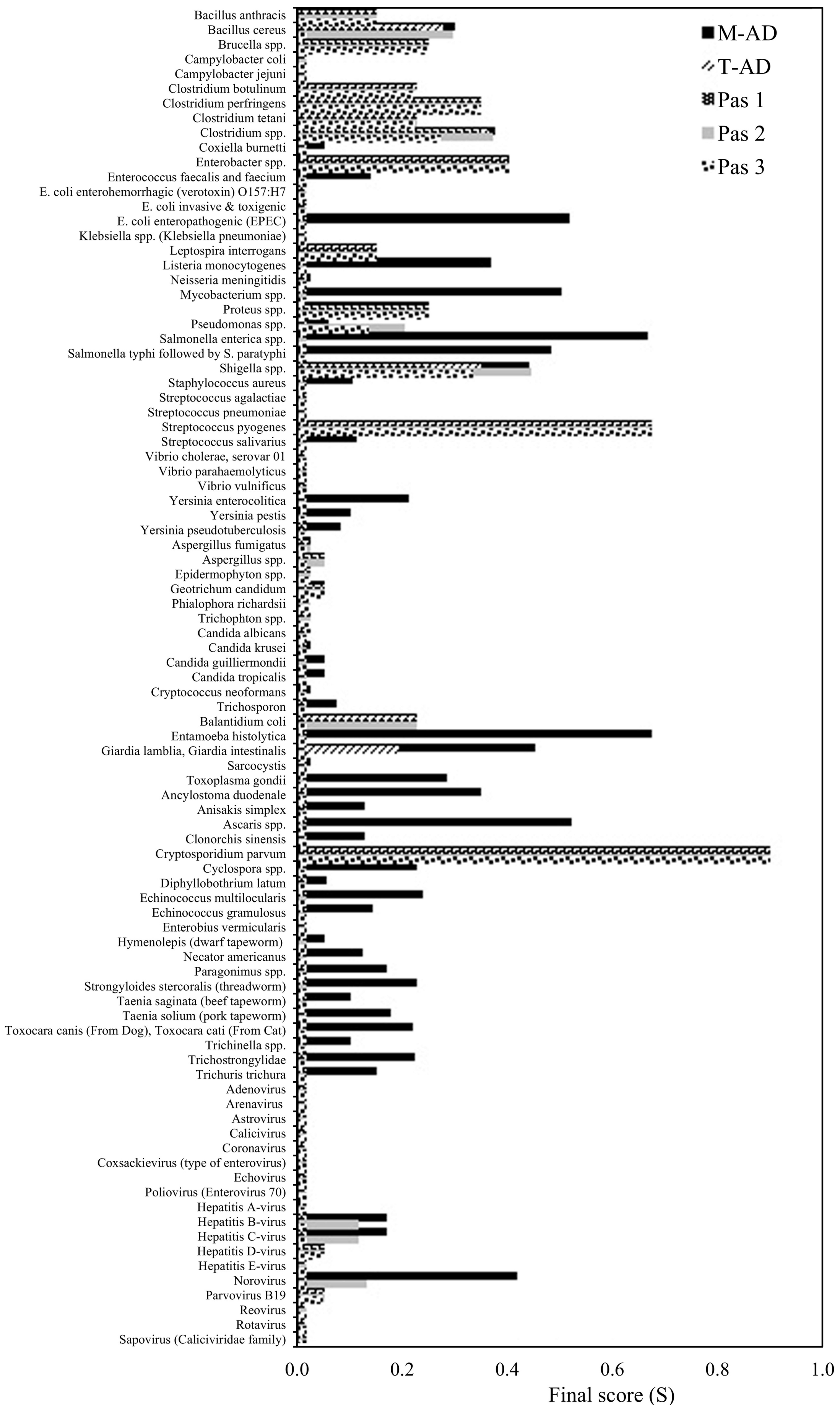


Figure 4

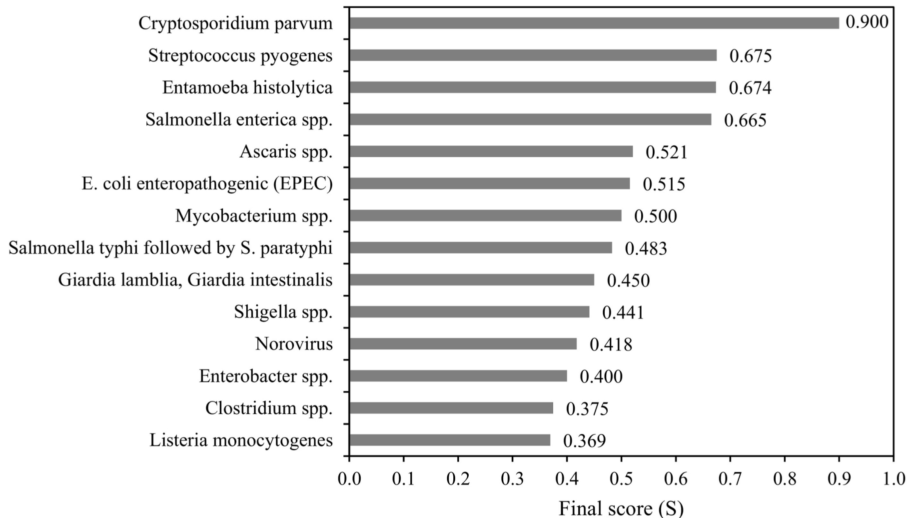


Figure 5

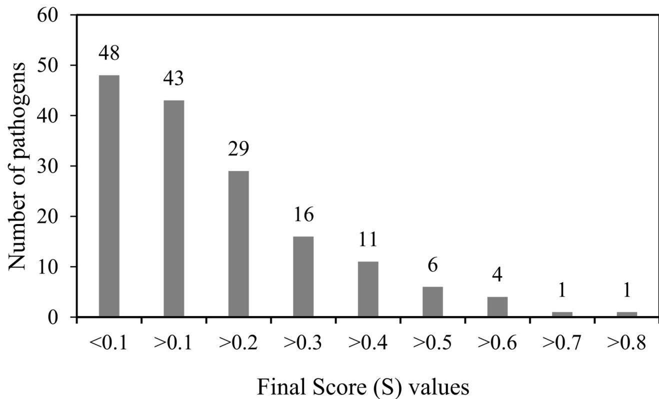
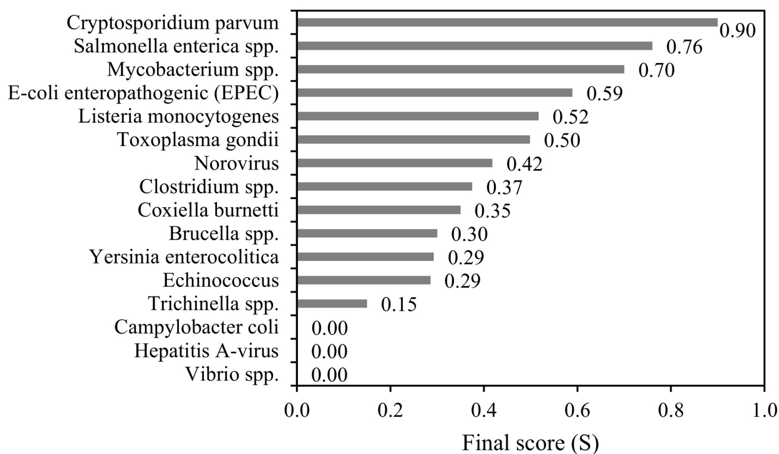
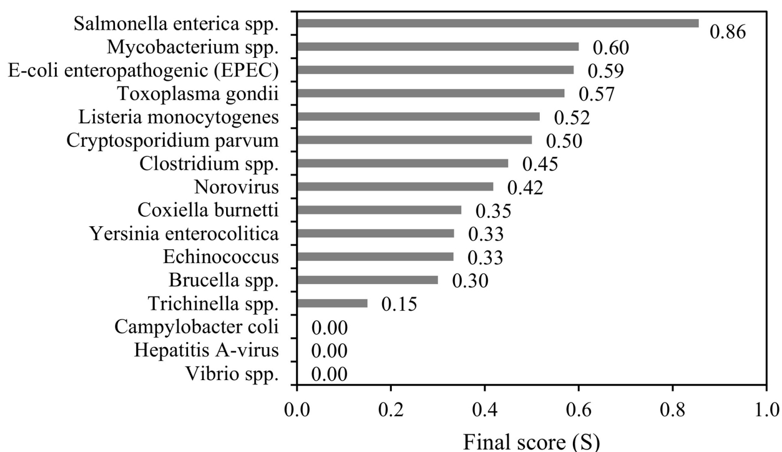


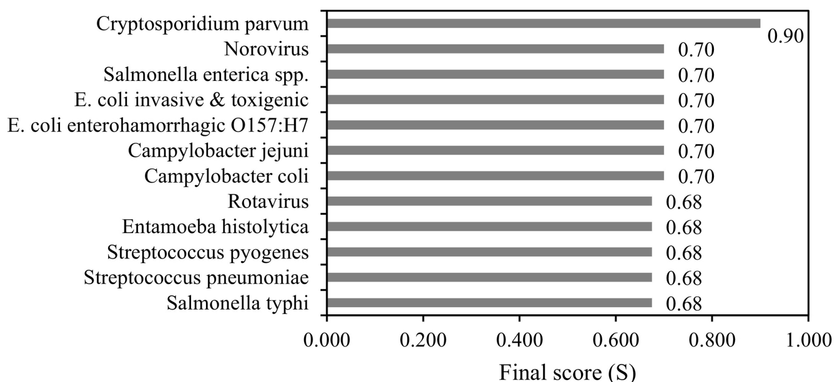
Figure 6



a) **Scenario A** **FOODIRE**



b) **Scenario B** **FOODEU**



c) **Scenario C** **RAWFYM&S**

Figure 7

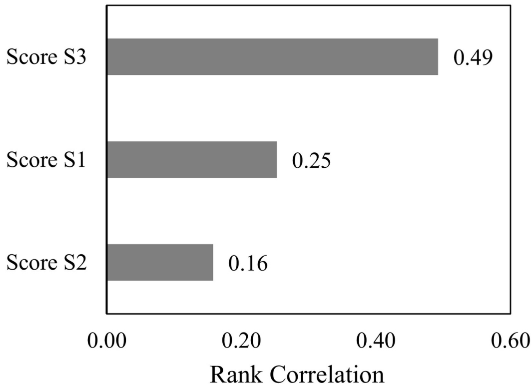


Figure 8