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A Bayesian inference approach to quantify average pathogen loads in farmyard manure and slurry using open-source Irish datasets

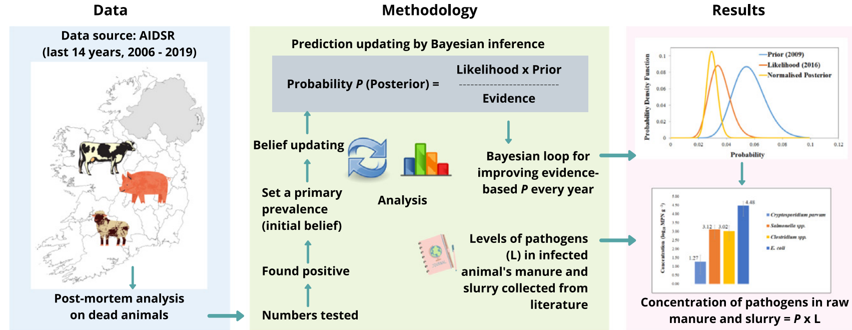
Rajat Nag [a,*], Bryan K. Markey [b], Paul Whyte [b], Vincent O'Flaherty [c], Declan Bolton [d], Owen Fenton [e], Karl G. Richards [e], Enda Cummins [a]

[a] University College Dublin, School of Biosystems and Food Engineering, Belfield, Dublin 4, Ireland
[b] University College Dublin, School of Veterinary Medicine, Belfield, Dublin 4, Ireland
[c] National University of Ireland Galway, School of Natural Sciences, Galway, Ireland
[d] TEAGASC, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland
[e] TEAGASC, Environment Research Centre, Johnstown Castle, County Wexford, Ireland

HIGHLIGHTS

- A Bayesian inference loop was used to estimate pathogen prevalence in animals.
- Both historical and recent data was captured.
- More accurate prediction of prevalence is possible with a Bayesian inference loop.
- Provides an initial estimate for pathogen levels in faeces
- These levels will help to integrate robust farm-to-fork risk assessments.

GRAPHICAL ABSTRACT



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ABSTRACT

Farm-to-fork quantitative microbial risk assessments (QMRA) typically start with a preliminary estimate of initial concentration ($C_{initial}$) of microorganism loading at farm level, consisting of an initial estimate of prevalence (P) and the resulting pathogen levels in animal faeces. An average estimation of the initial concentration of pathogens can be achieved by combining P estimates in animal populations and the levels of pathogens in colonised animals' faeces and resulting cumulative levels in herd farmyard manure and slurry (FYM&S). In the present study, 14 years of data were collated and assessed using a Bayesian inference loop to assess the likely P of pathogens. In this regard, historical and current survey data exists on P estimates for a number of pathogens, including *Cryptosporidium parvum, Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Salmonella* spp., *Clostridium* spp., *Campylobacter* spp., pathogenic *E. coli*, and *Listeria monocytogenes* in several species (cattle, pigs, and sheep) in Ireland. The results revealed that *Cryptosporidium* spp. has potentially the highest mean P (P_{mean}) (25.93%), followed by *MAP* (15.68%) and *Campylobacter* spp. (8.80%) for cattle. The P_{mean} of *E. coli* is highest (7.42%) in pigs, while the P_{mean} of *Clostridium* spp. in sheep was estimated to be 7.94%. $C_{initial}$ for *Cryptosporidium* spp., MAP., *Salmonella* spp., *Clostridium* spp., and *Campylobacter* spp. in cattle faeces were derived with an average of 2.69, 4.38, 4.24, 3.46, and 3.84 $\log_{10}$ MPN g $^{-1}$, respectively. Average $C_{initial}$ of *Cryptosporidium* spp., *Salmonella* spp., *Clostridium* spp., and *E. coli* in pig slurry was estimated as 1.27, 3.12, 3.02, and 4.48 $\log_{10}$ MPN g $^{-1}$,

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

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respectively. It was only possible to calculate the average $C_{initial}$ of *Listeria monocytogenes* in sheep manure as 1.86 log_{10} MPN g $^{-1}$. This study creates a basis for future farm-to-fork risk assessment models to base initial pathogen loading values for animal faeces and enhance risk assessment efforts.

1. Introduction

Animal manure is a by-product of animal production systems, including cattle, pig, and sheep. It has been used as a valuable soil improver and, more recently, as a potential feedstock for anaerobic digestion (AD) biogas systems (Nag et al., 2019). Anaerobic digestate is a valuable by-product of AD and can be applied to land as organic fertiliser, similar to raw farmyard manure and slurry (FYM&S). However, under both scenarios, there is concern regarding the pathogenic loading of animal manure (Longhurst et al., 2019), given it has been implicated in some high profile disease outbreak cases due to pathogens such as *Salmonella* spp., *Cryptosporidium* spp., pathogenic *E. coli*, and *Campylobacter* spp. (Nag et al., 2020b). In addition, the initial basis of many pathogen farm-to-fork risk assessments commences with an initial estimate of pathogen loading in faeces at the start of the chain, *i.e.* at farm level (Hutchison et al., 2004). This estimation usually evaluates prevalence (*P*) and resulting levels of the pathogen in animal faeces. This step has been identified as critically important in the sensitivity analysis of several farm-to-fork risk assessments (Longhurst et al., 2013), highlighting the need for approaches to improve bacterial loading estimates in animal faeces.

Table 1 shows the summarised results of studies worldwide reporting the presence of several target pathogens in raw FYM&S. These pathogens can be transmitted to humans by environmental pathways such as air, water, soil (food), and direct contact (Nag et al., 2019). From Table 1, it is clear that the *P* of colonisation in animals varies widely. It varies from country to country and from animal to animal (Perry and Grace, 2009; Tomley and Shirley, 2009). Gale et al. (2009) found that geographical boundaries and climate variation may influence animals' infection rates. Vaccination strategy also plays an important role in efforts to control certain zoonotic pathogens in animals (AFBI and DAFM, 2015). In Ireland, disease *P* for several pathogens in a wide range of animal species is reported annually in the All Island Disease Surveillance Report (AIDSR). The AIDSR is an evidence-based yearly publication (AFBI and DAFM, 2016) produced by the Department of Agriculture, Food and the Marine (DAFM, Republic of Ireland), and Agri-food and Biosciences Institute (AFBI, Northern Ireland) in collaboration with the Department of Agriculture, Environment and Rural Affairs, Animal Health and Welfare Northern Ireland, Marine Institute, the Irish Equine Centre and Animal Health Ireland. The AIDSR yearly report aims to provide industries with an early warning system for introducing exotic disease or the impact of a novel or emerging disease by tracking the pattern and frequency of diseases in farmed animals from year-to-year (AFBI and DAFM, 2017). The analysis includes both passive and active surveillance results but is largely based on the results of *post mortem* examination of faecal samples of fallen animals (with or without disease) with testing carried out in regional veterinary laboratories. The test-positive percentage of the faecal samples of the fallen animals is presented in Fig. 1. A significant drop has been observed in the last three or four years in the percentage positive cases of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Cryptosporidium* spp. in cattle and *E. coli* (strains not specified) in pigs and sheep. Also, it must be noted that from 2017 onwards, the data for Northern Ireland and Republic of Ireland (ROI) could be separated and therefore, only ROI data is presented from 2017 onwards.

There is both variability and uncertainty regarding the levels of pathogens present in manure from different species. This study tries to capture that uncertainty in Irish FYM&S samples using Bayesian inference techniques. Bayesian inference is a useful tool to combine current knowledge with historical survey data to get an overall evaluation of an outcome (von Westerholt and Butler, 2020). Bayesian can be used to determine the viral load in both sources and treated water more accurately (Varughese et al., 2018). Also, a Bayesian Modeling was explored to track microbial sources over the landscape (Wu, 2019). In so doing, the results of this work may be useful in characterising pathogen loads for use in future risk assessment studies.

This study's principal hypothesis was "*Bayesian Inference is a statistical technique that can be used to collate multiple yearly prevalence (P) surveys to give a more robust overarching indication of pathogen loads in FYM&S*". Hence, the overall aim of the study was to develop a Bayesian inference model to quantify average pathogen loads in FYM&S based upon several years of survey results which are combined

Table 1
Relevant studies on the presence of target pathogens in raw FYM&S.

Number	Pathogen name	Number tested (individual samples)			Found positive in fresh FYM&S (% positive)			Country	Reference
		Cattle	Pig	Sheep	Cattle	Pig	Sheep		
1	*Cryptosporidium parvum*	810	126	24	44 (5.4)	17 (13.5)	7 (29.2)	UK	(Hutchison et al., 2004)
		7	9	9	4 (57.1)	7 (77.8)	6 (66.7)	Australia	(Cox et al., 2005)
2	*Mycobacterium bovis*				(9, 46)[a]			Ireland	(Cotter et al., 1996)
							(4, 6)[a]	Australia	(Dhand et al., 2007)
								Bangladesh	(Reza et al., 2015)
								Canada	(Alexander et al., 1968)
		412,975	6714	10,000	16,519 (4.0)	330 (4.9)	12 (0.1)	Italy	(Amato et al., 2018)
3	*Salmonella* spp.	810	126	24	62 (7.7)	10 (7.9)	2 (8.3)	UK	(Hutchison et al., 2004)
4	*Listeria monocytogenes*	810	126	24	241 (29.8)	25 (19.8)	7 (29.2)	UK	(Hutchison et al., 2004)
		528			107 (20.3)			USA	(Nightingale et al., 2004)
5	*Clostridium* spp.	104	100	100	0 (0.0)	0 (0.0)	1 (1.0)	UK	(Salf and Brazier, 1996)
						(13, 54.5)[a]		Spain	(Alvarez-Perez et al., 2009)
		196			44 (22.4)			Germany	(Krüger et al., 2012)
		9	9	9	2 (22.2)	9 (100.0)	2 (22.2)	Australia	(Cox et al., 2005)
6	*E. coli* (O157)	810	126	24	107 (13.2)	15 (11.9)	5 (20.8)	UK	(Hutchison et al., 2004)
7	*Campylobacter* spp.	810	126	24	104 (12.8)	17 (13.4)	5 (20.8)	UK	(Hutchison et al., 2004)

[a] Minimum and maximum values.

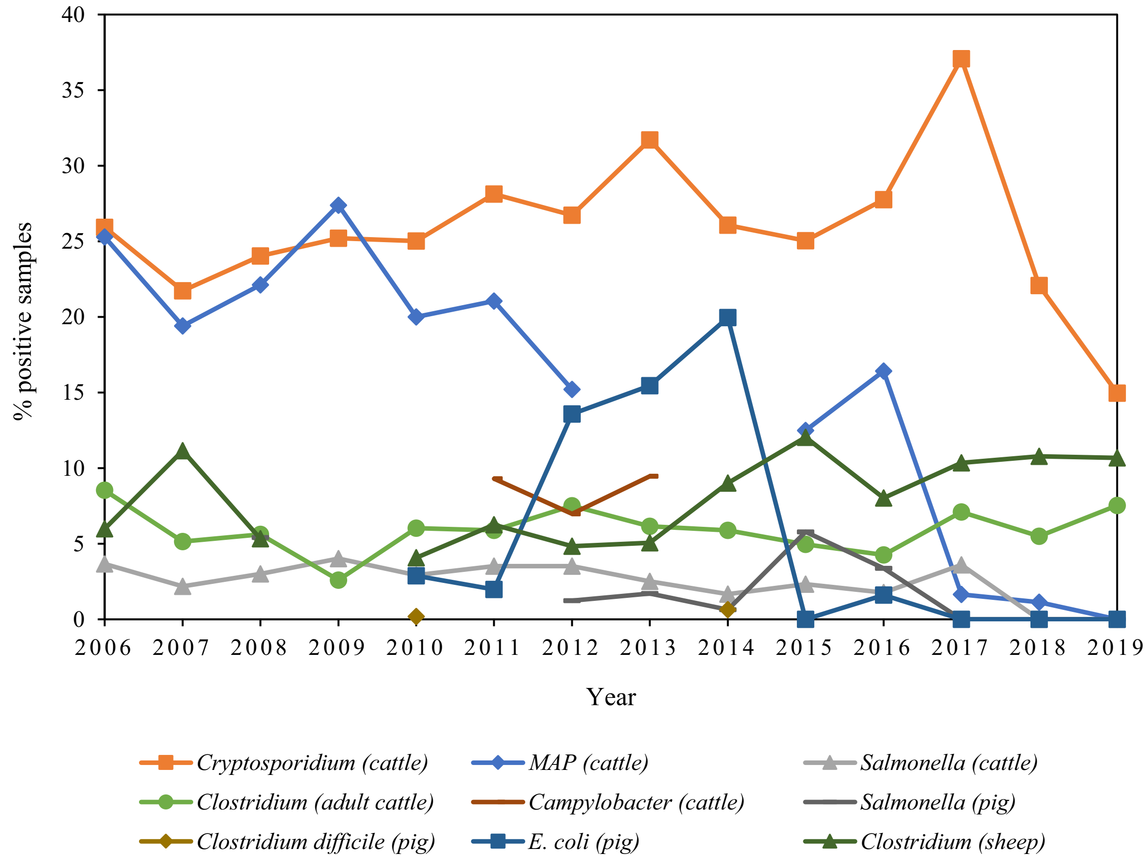

Fig. 1. The variation of % positive samples over 14 years of the observed period of study; source AIDSR reports 2007 to 2019; 2017 onwards, the data for Northern Ireland and Republic of Ireland (ROI) could be separated, and therefore, only ROI data is presented from 2017 onwards.

in a Bayesian loop to get a final posterior probability distribution representing the most likely levels of these pathogen loads in animal faeces. This approach can be critical as a first step towards developing a quantitative microbial risk assessment (QMRA) for these pathogens.

2. Materials and methods

The probabilistic study was based on the following steps.

- Estimating the *P* of target pathogens causing mainly enteric disease in animals, among various animal species, with a Bayesian inference loop.
- Collation of literature-based data regarding the range of pathogen concentrations in faeces of infected animals.
- Combine I and II above to get the overall average range of initial concentration ($C_{initial}$) in FYM&S (Fig. 2) following a similar approach by Longhurst et al. (2013).

2.1. Raw data collection from AIDSR (from 2006 to 2019)

The AIDSR reports collate data relating to: ‘primarily in scanning (passive) surveillance by gathering data from *post mortem* and clinical sample submissions’ (AFBI and DAFM, 2018). The data provides an insight into disease incidence trends and the principal causes of mortality on Irish farms, thereby informing decision-making relevant to disease control at a national level (AFBI and DAFM, 2018). It is an all-island database disease surveillance report. Data for the years 2006 to 2019 are tabulated in Table 2 and subsequently used in the model to represent the number tested (N) and the number positive (s) as represented in the model framework (Fig. 2).

The frequency of pathogens (*Cryptosporidium parvum*, *Salmonella* spp.) identified in both *post mortem* submissions and faecal clinical samples from calves less than one month of age submitted to DAFM laboratories was collected from AIDSR reports. The *P* reduces in adult animals which also raises the concern of an overestimation. However, the pessimistic approach is very common in risk assessment studies as a worst-case scenario (Hannon et al., 2017). Johne's disease, which is caused by infection with MAP, is notifiable, and although primarily affecting cattle in Ireland, it can also occur in sheep and goats (Manning and Collins, 2001). Data for *Clostridium* spp. (*C. perfringens, C. botulinum, C. novyi, C. sordellii, C. chauvoei, C. sordellii, C. septicum*) was available for different age groups such as less than 1 month, 1 to 5 months, 6 to 12 months, over 12 months and the 14-year average per cent positive was reported as 1.8, 4.7, 9.0, and 5.6%, respectively.

The average percentage positive (5.3%) of the entire population (cattle) matched closely with the average per cent positive of adult cattle (5.6%); therefore, data for adult cattle was considered as representative for the *P* of the clostridial disease in cattle of all ages. The target pathogens found to cause diseases in pigs were *Cryptosporidium parvum* (only one year of data for 2010), *Salmonella* spp., *Clostridium difficile*, and *E. coli* (strains not specified). *Salmonella* is frequently carried in pigs but rarely causes a disease; however, pigs remained an important contributor to human salmonellosis, responsible, in some states, for up to 56% of the confirmed human cases (Evangelopoulou et al., 2015). *E. coli* was reported as the most frequently reported disease of chickens worldwide and is often responsible for carcase condemnation at slaughter (AFBI and DAFM, 2011). Clostridial disease was stated as the most frequent disease in sheep. Where only one-year data is available, a beta (β) distribution was applied instead of Bayesian as the latter requires at least two years of data, one for the prior probability and another to calculate the new probability (posterior).

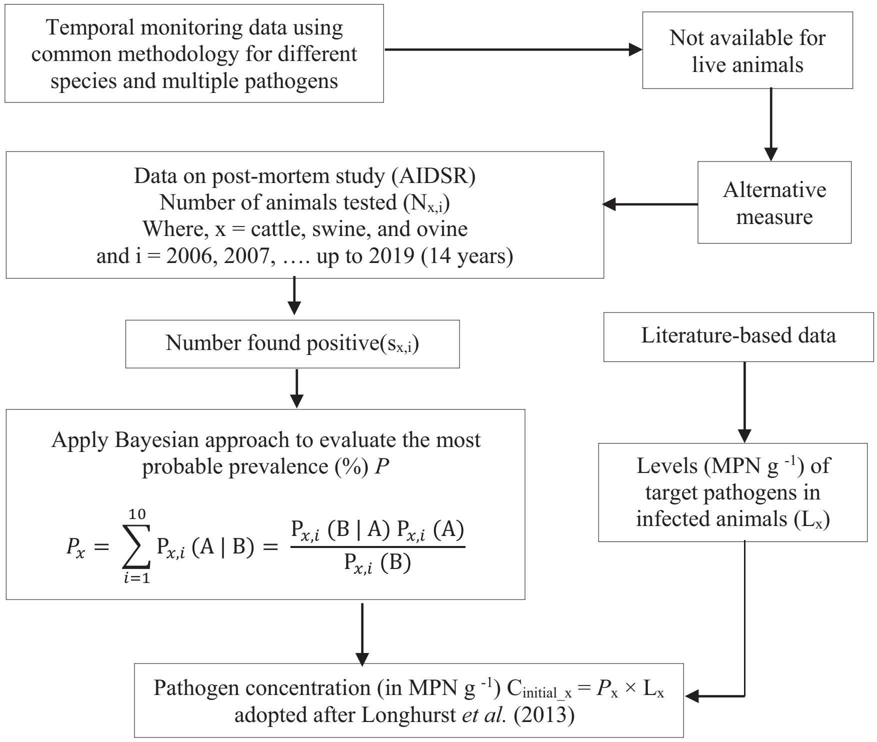

Fig. 2. A schematic flow diagram of the model.

2.2. Bayesian analysis of data

Bayesian inference is a powerful statistical technique based on Bayes' theorem for using data to improve one's estimate of a parameter (Vose, 2009). There are three steps involved in Bayesian analysis (Vose, 2009);

- determining a prior estimate of a parameter in the form of confidence distribution
- finding an appropriate likelihood function for the observed data
- calculating the posterior estimate (which is a revised estimation) by combining the prior and likelihood function, then normalising the estimate so that the final result becomes a true distribution of the parameter.

Bayesian analysis is treated as the best estimate of statistical distribution, given prior knowledge of the distribution (Fontana et al., 2009). The Bayesian method is based on Bayes' theorem (1763). The theorem states that,

$$P(A \mid B) = \frac{P(B \mid A)\, P(A)}{P(B)} \tag{1}$$

where A and B are events and $P(B) \neq 0$.

$P(A \mid B)$ is a conditional P: the likelihood of event A occurring given that B is true.
$P(B \mid A)$ is also a conditional P: the likelihood of event B occurring given that A is true.
$P(A)$ and $P(B)$ are the probabilities of observing A and B independently of each other (known as the marginal P).
Often, for some partition {Aj} of the sample space, the event space is given or conceptualised in terms of $P(Aj)$ and $P(B \mid Aj)$. It is then useful to compute $P(B)$ using the law of total P as

$$P(B) = \sum_{j=1}^{n} P(B \mid Aj)\, P(Aj) \tag{2}$$

Therefore, the extended form of the theorem states that if A and B are two events, the conditional P that A will occur given that B has occurred is denoted $P(Ai \mid B)$ and can be expressed as

$$P(Ai \mid B) = \frac{P(B \mid Ai)\, P(Ai)}{\sum_{j=1}^{n} P(B \mid Aj)\, P(Aj)} \tag{3}$$

In Bayesian inference, Bayes' theorem is presented as

$$f(\theta \mid x) = \frac{\pi(\theta)\, l(x \mid \theta)}{\int(\theta)\, l(x \mid \theta)\, d\theta} \tag{4}$$

where

$\pi(\theta)$ is the 'prior distribution', a density function of the prior belief about the parameter value θ before observing data x.
$l(x \mid \theta)$ is the 'likelihood function', which is the calculated P of randomly observing the data x for a given value of θ.
$f(\theta \mid x)$ is the 'posterior distribution' representing our knowledge about θ after observing the data x and given our opinion of the value of θ before x was observed.
The denominator in Eq. (4) normalises the $\pi(\theta)$, so the total area equals 1 (Vose, 2009). Bayesian inference mathematically describes the learning process; the model starts with an opinion, and then the opinion in the model is modified when presented with additional evidence. Several pieces of evidence are presented (*e.g.* annual surveillance data); this can be incorporated using a Bayesian Loop to give increased confidence about the output based upon a combination of all the evidence presented (Fontana et al., 2009).

In our case, first, a range of trials (t) was selected, say 100; next, the range of probability (P) was chosen according to the number of t; here 0, 0.01, 0.02 … to 0.99 (total 100 numbers). The data for 2006, which is denoted as $(i\text{-}1)^{th}$ year in Eq. (5), was selected as the prior knowledge for the model. Prior function for 2007 was calculated with Eq. (5), where 'i', 's', 'n' and 'P' represent the year 2007, number of test-positive (success), number of samples tested and probability, respectively. This *BINOMDIST*

Table 2
Summarised data for 'dead or fallen' animals extracted from AIDSR data (from 2006 to 2019 inclusive).

Pathogen name	Year												
	2006	2007		2008		2009		2010		2011		2012	
	Positives	Tested	Positives	Tested	Positives	Tested	Positives	Tested	Positives	Tested	Positives	Tested	Positives
Cryptosporidium parvum (cattle)	771	2563	557	2988	718	3209	809	3157	790	3670	1032	4637	1239
MAP (cattle)	43	304	59	416	92	376	103	410	82	632	133	941	143
Salmonella spp. (cattle)	109	2571	56	2988	90	3209	129	3157	92	3670	129	4637	163
Clostridium spp. (Adult cattle)	18	253	13	267	15	500	13	564	34	543	32	572	43
Campylobacter spp. (cattle)										3163	294	4386	306
Cryptosporidium parvum (pig)								519	4				
Salmonella spp. (pig)				406	22							162	2
Clostridium difficile (pig)								519	1				
E. coli (strains not specified) (pig)								519	15	355	7	162	22
Listeria monocytogenes (sheep)	12												
Clostridium spp. (sheep)	19	242	27	263	14			737	30	832	52	1241	60

Table 2 *(continued)*

Pathogen name	Year														Average % positive
	2013		2014		2015		2016		2017		2018		2019		
	Tested	Positives	Tested	Positives	Tested	Positives	Tested	Positives	Tested	Positives	Tested	Positives	Tested	Positives	
Cryptosporidium parvum (cattle)	2706	858	2658	693	4668	1169	5000	1388	863	320	1871	413	1370	205	25.8
MAP (cattle)					176	22	1822	299	550	9	527	6			16.6
Salmonella spp. (cattle)	2706	68	2645	44	4674	109	5000	88	863	31					3.7
Clostridium spp. (Adult cattle)	618	38	408	24	484	24	494	21	550	39	527	29	505	38	5.8
Campylobacter spp. (cattle)	4206	398									1644	146	1164	136	9.2
Cryptosporidium parvum (pig)															0.6
Salmonella spp. (pig)	233	4	466	3	121	7	622	21							2.8
Clostridium difficile (pig)			466	3											0.4
E. coli (strains not specified) (pig)	233	36	466	93	121	0	622	10							7.8
Listeria monocytogenes (sheep)															6.0
Clostridium spp. (sheep)	1126	57	887	80	905	109	1844	148	1315	136	798	86	553	59	7.9

Table 3
Parameters used for β distributions.

Target pathogens	N	s	α	α1
Cryptosporidium spp. in pigs	519	4	5	516
Listeria monocytogenes in sheep	192	12	13	181

excel-function calculates *P* of exactly 's' in (i-1) th year out of 'n' in (i-1) th year.

$$\pi(\theta)_i = BINOMDIST\left[s\ in\ (i{-}1)^{th}\ year, N\ in\ (i{-}1)^{th}\ year, P, FALSE\right] \quad (5)$$

Next, the likelihood $l(x \mid \theta)_n$ for the i^{th} year was calculated with the same *BINOMDIST* function (Eq. (6)) but based on the data for the i^{th} year; where 's', 'N' and '*P*' represent several positive samples (successes), number of samples tested, and probability, respectively. Similar to Eq. (5), this *BINOMDIST* function calculates *P* of exactly s in i th year out of N in i th year.

$$l(x \mid \theta)_i = BINOMDIST\left(s_{i^{th}\ year}, N_{i^{th}\ year}, P, FALSE\right) \quad (6)$$

A posterior function for the i th year was calculated as the multiplication of prior (i th year) and likelihood (i th year) (Eq. (7)) which can be further used as a prior function for the (i + 1) th year data (Vose, 2009); so the repetitive calculation was done from 2006 to 2019 to get the posterior function for 2019. Finally, the posterior value for the latest year (2019) was normalised by Eq. (8), where *P*t stands for the individual probability of unique trial t.

$$f(\theta \mid x)_i = \pi(\theta)_i \times l(x \mid \theta)_i \quad (7)$$

$$f(\theta \mid x)_{2019,normalised} = \frac{f(\theta \mid x)_{2019}\ for\ P_t}{\sum_{t=0}^{0.99} f(\theta \mid x)_{2019}\ for\ P_t} \quad (8)$$

The prevalence (*P*) can be estimated from the *P vs* $f(\theta \mid x)_{normalised}$ graph. In the absence of a complete dataset from 2006 to 2019, when data is available for only one year, a β distribution was used to capture the uncertainty around the probability estimation. The β distribution is characterised by 2 parameters (Banmairuroy et al., 2014), alpha and beta, as shown in Eq. (9).

$$P = Beta\ (\alpha, \beta) \quad (9)$$

The alpha parameter is calculated by alpha = s + α, and the beta parameter is calculated by beta = N - s + β, where 's' is the positive samples (success) trial(s) in the identical 'n' trials (sample size) of a binomial process as shown in Eq. (11). In this study, β distribution was used for *Cryptosporidium* in pig (the reference year 2010; *N* = 519 tested and s = 4 found positive) and *Listeria monocytogenes* in sheep (the reference year 2006; *N* = 192 tested and s = 12 found positive).

$$P = Beta\ (s + \alpha, N - s + \beta) \quad (10)$$

If the prior distribution is an uninformed prior and the likelihood function is binomial distribution in Bayesian inference, this notation is a posterior distribution. If beta (1,1) distribution, which is equivalent to uniform (0,1) distribution, is an uninformed prior (FAO/WHO, 2008), alpha and beta will be replaced by 1 as shown in Eq. (3).

$$P_1 = Beta\ (s + 1, N - s + 1) \quad (11)$$

This approach is possible since the beta distribution is a conjugate distribution to the binomial likelihood function in Bayesian inference (Banmairuroy et al., 2014). Therefore, parameters α and α1 become s + 1 and N - s + 1, respectively (FAO/WHO, 2008). The α and α1 for *Cryptosporidium* (in pigs) and *Listeria monocytogenes* (in sheep) are displayed in Table 3. RiskBeta(α and α1) function was used in @RISK 7.5, and 100,000 iterations were performed to get the minimum, mean, maximum and standard deviation (σ) values.

2.3. Levels of pathogens (raw data collection)

Data on the likely level of pathogens in infected animals' faeces was collated from available scientific literature for EU member states. The data extracted for levels of pathogens in raw faeces of infected animals are presented in Table 4. A uniform distribution was used to capture the data variability for the concentrations (Table 5). It was observed that cattle and pig slurry is a major source of the target pathogens. The average level of contamination in FYM&S ($C_{initial}$) in an infected animal's manure was calculated by multiplying the *P* of the disease in animals and reported levels of the aetiological pathogen in the faeces of infected animals.

3. Results and discussion

Typical annual (from 2007 to 2019) likelihood values of *Cryptosporidium parvum* (cattle) is presented in Fig. 3. The probability density

Table 4
Estimated mean levels of pathogens in fresh FYM&S.

Pathogen name	Maximum Levels of pathogens in fresh FYM&S			Unit[a]	Country	Reference
	Cattle	Pig	Sheep			
Cryptosporidium parvum	2.7×10^2 to 3.5×10^3	3×10^2 to 3.6×10^3	5.3×10^1 to 2.5×10^2	Oocysts g^{-1}	UK	(Hutchison et al., 2004)[b]
Mycobacterium bovis	6.5×10^3			CFU ml^{-1}	Ireland	(Scanlon and Quinn, 2000)
MAP	3×10^5			CFU g^{-1}	USA	(Bonhotal et al., 2011)
Salmonella spp.	3.9×10^4 to 5.8×10^5	9.6×10^3 to 7.8×10^4	1.1×10^3 to 2×10^3	CFU g^{-1}	UK	(Hutchison et al., 2004)[b]
Listeria monocytogenes	1.5×10^4 to 4.2×10^5	4.6×10^4 to 9.7×10^5	4.5×10^2 to 1.7×10^3	CFU g^{-1}	UK	(Hutchison et al., 2004)[b]
Clostridium spp.	$10^{4.95}$	$10^{5.28}$		CFU g^{-1}	Italy	(Costa et al., 2017)
		1.0×10^5 to $1.0 \times 10^{5.5}$		CFU g^{-1}	Ireland	(Mccarthy et al., 2013)
	1.0×10^4 to $1.0 \times 10^{4.6}$			CFU g^{-1}	Sweden	(Bagge et al., 2005)
Clostridium spp. (combined)	1.0×10^4 to $1.0 \times 10^{4.95}$	1.0×10^5 to $1.0 \times 10^{5.5}$		CFU g^{-1}		
E. coli	$5.1 \times 10^4 \pm 4.5 \times 10^4$	$3 \times 10^4 \pm 7.1 \times 10^3$		CFU g^{-1}	France	(Jaffrezic et al., 2011)
		$1.0 \times 10^{3.8}$ to $1.0 \times 10^{5.5}$		CFU g^{-1}	Ireland	(Mccarthy et al., 2013)
E. coli (combined)	0.6×10^4 to 9.6×10^4	$1.0 \times 10^{3.8}$ to $1.0 \times 10^{5.5}$		CFU g^{-1}		
E. coli O157	2.9×10^6 to 2.6×10^8	6.9×10^4 to 7.5×10^5	1.1×10^4 to 4.9×10^4	CFU g^{-1}	UK	(Hutchison et al., 2004)[b]
Campylobacter spp.	7.6×10^3 to 1.5×10^5	1.9×10^3 to 1.5×10^4	8.6×10^2 to 2.1×10^3	CFU g^{-1}	UK	(Hutchison et al., 2004)[b]

[a] The specific gravity of slurry was considered as S.G. = (% dry matter +255.8) ÷ 255.46 (Tunney and Molloy, 1975). Considering mean % dry matter as 7.6 (Nolan et al., 2018), S.G. is calculated as 1.031. So, the conversion factor for CFU ml^{-1} to CFU g^{-1} of total solid is considered as 0.97. Alternatively, to be on the safer side, CFU ml^{-1} = CFU g^{-1}.
[b] The lower limit of the concentration is not mentioned in (Hutchison et al., 2004); the range is selected based on the mean and maximum limit being on the safer side.

Table 5
Adopted distributions for levels of pathogens in fresh FYM&S, which is used in this research.

Pathogen name	Different animals			Unit
	Cattle	Pig	Sheep	
Cryptosporidium parvum	Uniform(2.7×10^{2}, 3.5×10^{3})	Uniform(3×10^{2}, 3.6×10^{3})		Oocysts g^{-1}
MAP	Uniform(6.5×10^{3}, 3×10^{5})			CFU g^{-1}
Salmonella spp.	Uniform(3.9×10^{4}, 5.8×10^{5})	Uniform(9.6×10^{3}, 7.8×10^{4})		CFU g^{-1}
Listeria monocytogenes			Uniform(4.5×10^{2}, 1.7×10^{3})	CFU g^{-1}
Clostridium spp.	Uniform(1×10^{4}, $1 \times 10^{4.95}$)	Uniform(1×10^{5}, $1 \times 10^{5.5}$)		CFU g^{-1}
E. coli		Uniform($1.0 \times 10^{3.8}$, $1.0 \times 10^{5.5}$)		CFU g^{-1}
Campylobacter spp.	Uniform(7.6×10^{3}, 1.5×10^{5})			CFU g^{-1}

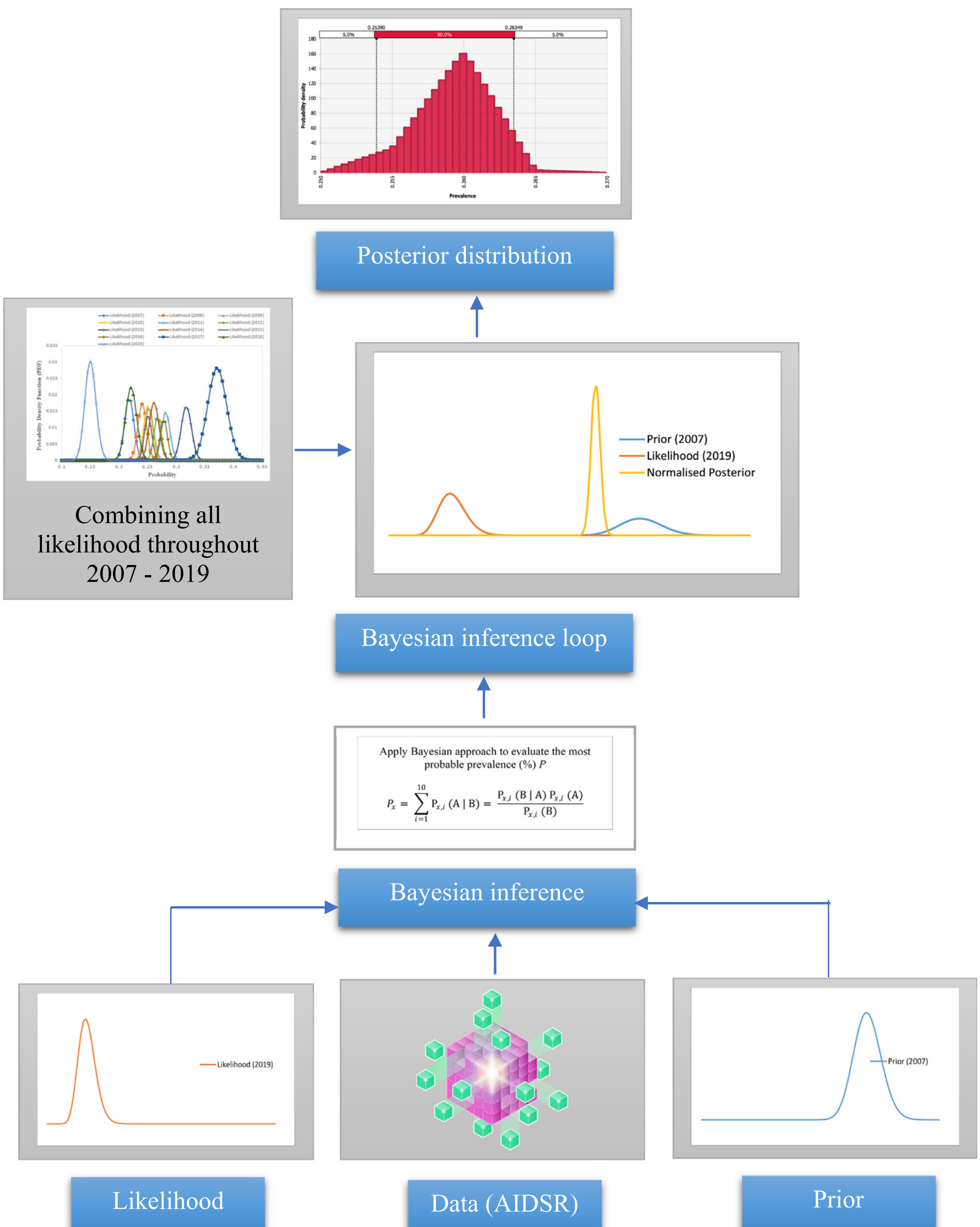

Fig. 3. A typical representation of how posterior distribution is obtained from yearly likelihood and prior knowledge combined by Bayesian inference loop.

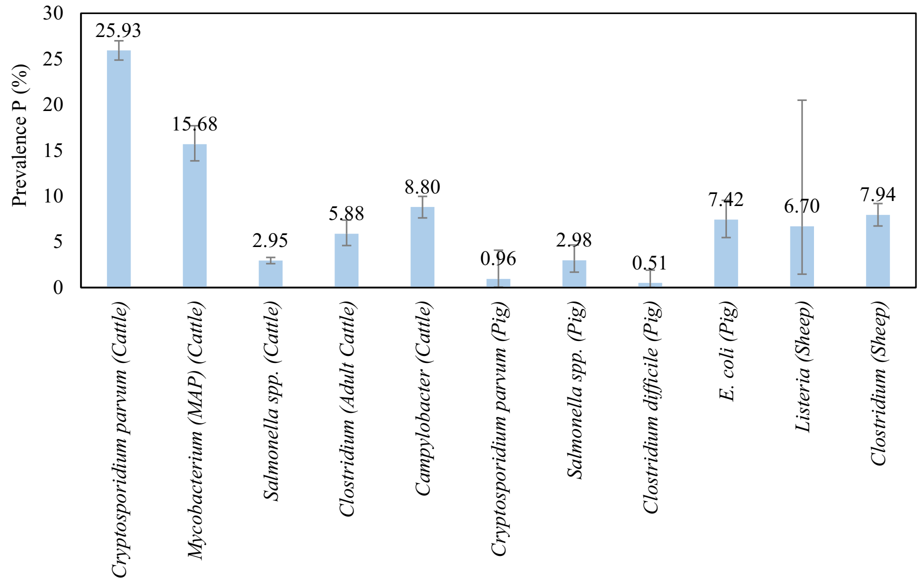

Fig. 4. Estimated Prevalence (P) of selected pathogens in animals in Ireland using Bayesian analysis. Note: Data for *Cryptosporidium* spp. in pig was available only for 2010; hence Beta distribution was applied instead of Bayesian.

function (PDF) is presented on the Y-axis, whereas X-axis data represent *P*. The highest probability was observed in 2017 (37.5%), and gradually it dropped towards 2019 (15%). The normalised posterior function was obtained combining prior and likelihood distributions with a Bayesian inference loop (Figs. 2 and 3). Also, the literature states that Bayesian statistics is very popular in QMRA to account for left-censored microbial abundance distributions (Daniels et al., 2014; Jamal et al., 2020; Mathai et al., 2020). In cattle, the highest mean *P* (P_{mean}) was observed for *Cryptosporidium* spp. (25.93%); followed by MAP (15.68%), *Campylobacter* spp. (8.80%), *Clostridium* spp. (5.88%), and *Salmonella* spp. (2.95%). The P_{mean} of *E. coli*, *Salmonella* spp., and *Clostridium difficile* were 7.42%, 2.98%, and 0.51%, respectively in pigs whereas, the P_{mean} in sheep for *Listeria* spp. and *Clostridium* spp. was found to be 6.70% and 7.94%, respectively (Fig. 4). Data for *Cryptosporidium* spp. in pigs and *Listeria monocytogenes* in sheep was available only for 2010 and 2006, respectively; hence β distribution was applied instead of Bayesian; consequently, the error for the *Cryptosporidium* spp. in pig was moderate, and the probability of *Listeria monocytogenes* spp. *in* sheep showed the widest uncertainty compared to other values in Fig. 4.

A comparison between literature-based *P* and the estimated P_{mean} (%) of the pathogen in animals from Bayesian analysis is presented in Table 6. Spatial distribution of 944 Irish herds showed the *P* of *Cryptosporidium* spp. in cattle ranged from 18 to 35% in Ireland (North 31%, Northwest 34%, Midwest 35%, Southwest 20%, Southeast 18%, Northeast 25%) (de Waele et al., 2013). Mirhashemi et al. (2016) also reported that the overall *P* of *Cryptosporidium* spp. oocysts in animal samples ranged from 21.5–22.5% in cattle and 14–16.5% in sheep based on 2009–2010 data. The median posterior estimate for MAP among dairy herds was 28% (95% interval: 0.23% - 0.34%) (McAloon et al., 2016). *Salmonella* spp. was isolated from 2% of faecal, 2% of the rumen and 7.6% of carcass samples (McEvoy et al., 2003). Based on beef abattoir-studies, Madden et al. (2007) estimated the *P* of *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter* spp. as 3, 4.8, and 24.8%, respectively. Moschonas et al. (2009) estimated the *P* of *Clostridium* spp. in cattle as 2.1%. According to Table 6, the estimated P_{mean} of *Cryptosporidium* spp. and *Salmonella* spp. supports previously reported values. However, the reported range of MAP was higher than the estimated value, whereas the reported *P* of *Campylobacter* spp. was very high compared to the estimated value, which requires further investigation. This study also

Table 6
A comparison between literature-based reported prevalence (P) and estimated P_{mean} (%) of the pathogen in animals from Bayesian analysis.

Number	Pathogen name	Study in Republic of Ireland or Northern Ireland	Animal	Reported prevalence (*P*)	Estimated P_{mean} (%)		
		Reference		(%)	Cattle	Pig	Sheep
1	*Cryptosporidium* spp.	(de Waele et al., 2013)	Cattle	18–35	26.9	0.96	
		(Mirhashemi et al., 2016)	Cattle	21.5–22.5			
		(Mirhashemi et al., 2016)	Sheep	14–16.5			
2	MAP	(McAloon et al., 2016)	Cattle	28 (23–34)	15.6		
3	*Salmonella* spp.	(McEvoy et al., 2003)	Cattle	2–7.6	2.95	2.98	
		(Madden et al., 2007)[a]	Cattle	3			
4	*Listeria monocytogenes*	(Madden et al., 2007)[a]	Cattle	4.8			6.70
5	*Clostridium* spp.	(Moschonas et al., 2009)[c]	Cattle	2.1	5.88	0.50	7.94
6	*E. coli*	(O'Brien et al., 2005)[b]	Cattle	7.3		7.41	
7	*Campylobacter* spp.	(Madden et al., 2007)[a]	Cattle	24.8	8.80		

Note: blank cells denote no data.
[a] Slaughterhouse study.
[b] *E. coli* O157.
[c] Based on meat study: samples from the main meat conveyor belt.

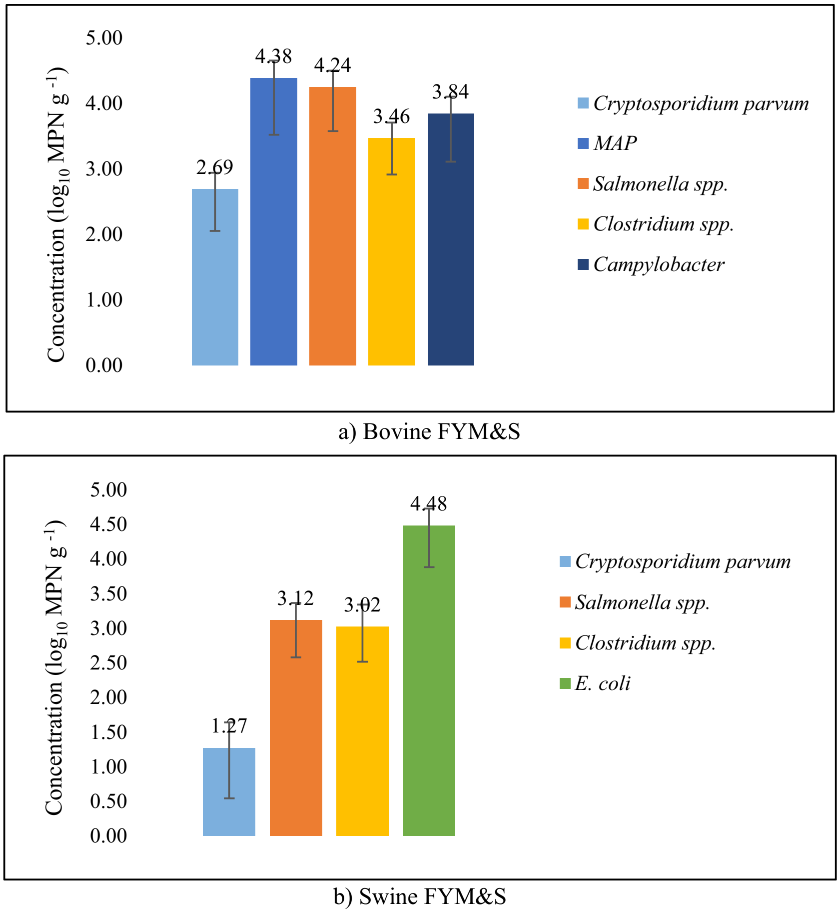

Fig. 5. The estimated initial concentration of pathogens ($C_{initial}$) in raw FYM&S.

found that the estimated P_{mean} of *Clostridium* spp. is higher than the previously reported value.

This research found that the simulated mean $C_{initial}$ of *Cryptosporidium* spp., MAP, *Salmonella* spp., *Clostridium* spp., and *Campylobacter* spp. in cattle manure was 2.69, 4.38, 4.24, 3.46, and 3.84 $\log_{10}$ MPN g $^{-1}$, respectively. The simulated mean $C_{initial}$ of *Cryptosporidium* spp., *Salmonella* spp., *Clostridium* spp., and *E. coli* in swine slurry was evaluated as 1.27, 3.12, 3.02, and 4.48 $\log_{10}$ MPN g $^{-1}$, respectively (Fig. 5). The simulated mean $C_{initial}$ of *Listeria monocytogenes* was evaluated as 1.86 $\log_{10}$ MPN g $^{-1}$ in sheep manure with a negative and positive error (based on 95 confidence interval) of 2.68 $\log_{10}$ MPN g $^{-1}$ and 3.17 $\log_{10}$ MPN g $^{-1}$, respectively. Overall observation found that $C_{initial}$ of *Cryptosporidium* spp., *Salmonella* spp., and *Clostridium* spp. in cattle is higher than in pig slurry. A summary of this research's key findings highlighting the simulated prevalence and levels of pathogens in FYM&S is collated in Table 7.

3.1. Assumptions, limitations and future work

a) The concentrations of target pathogens in FYM&S of healthy animals were assumed to be zero, whereas, in fact, 'healthy' animals may have subclinical infections. Hence this value could be underestimated concerning the level of pathogens contributed by the 'healthy' population.
b) A few pathogens such as *Cryptosporidium parvum* and *Salmonella* spp. are very common in juvenile animals, but the *P* reduces in

Table 7
Summary of simulated prevalence and levels of pathogens in FYM&S.

Animal	Pathogen	Prevalence (*P*) (%)		Initial concentration ($C_{initial}$) in manure/slurry ($\log_{10}$ MPN g $^{-1}$)	
		Mean	Confidence interval (5th - 95th)	Mean	Confidence interval (5th - 95th)
Cattle	*Cryptosporidium* spp.	25.93	(25.38–26.34)	2.69	(2.05–2.94)
Cattle	MAP	15.68	(14.85–16.51)	4.38	(3.52–4.65)
Cattle	*Salmonella* spp.	2.95	(2.79–3.10)	4.24	(3.57–4.50)
Cattle	*Clostridium* spp.	5.88	(5.45–5.88)	3.46	(2.91–3.70)
Cattle	*Campylobacter* spp.	8.80	(8.24–9.31)	3.84	(3.11–4.10)
Pig	*Cryptosporidium* spp.	0.96	(0.37–1.75)	1.27	(0.54–1.64)
Pig	*Salmonella* spp.	2.98	(2.38–3.63)	3.12	(2.58–3.36)
Pig	*Clostridium* spp.	0.51	(0.19–0.93)	3.02	(2.52–3.34)
Pig	*E. coli*	7.42	(6.57–8.30)	4.48	(3.88–4.73)
Sheep	*Listeria monocytogenes*	6.70	(4.02–9.87)	1.86	(1.47–2.12)
Sheep	*Clostridium* spp.	7.94	(7.36–8.44)	–	–

adult animals, raising the concern of an overestimation. Therefore, more age-based classified data for animals may improve the current estimate; however, the pessimistic approach is very common in risk assessment studies as a worst-case scenario (Hannon et al., 2017).

c) A significant drop has been observed in the last three or four years in the percentage positive cases of MAP and *Cryptosporidium* spp. in cattle and *E. coli* in pigs and sheep. A Bayesian approach is used to estimate a parameter that is assumed to be relatively constant. Therefore, it is not a good tool to predict trends. For example, the drop in the test-positive cases in recent years could be linked to vaccination efforts that may not be captured; however, the methodology presented in this research can be considered a worst-case estimate.
d) The datasets used are largely based on passive surveillance and carcase submission. As a result, the *P* figures for conditions that do not result in high mortality levels may be underestimated.
e) Increased data completeness will improve the estimation of *P*; therefore, results can be updated every year.
f) As a recommendation, pathogenic *E. coli* (O157:H7) should be monitored and included in AIDSR research along with *E. coli* K99, which causes calf diarrhoea in cattle. *E. coli* O157:H7 is a growing concern in Ireland, particularly associated with major outbreaks in Ireland (Nag et al., 2020a).
g) Bayesian inference can be used in the future in cases where a beta distribution is used presently; for example, *Cryptosporidium* spp. in pigs and *Listeria monocytogenes* in sheep, as the recent likelihood result was not available.
h) Some of the *Clostridium* spp. are common in the environment, and *P* figures can be exaggerated if the faecal samples are not carefully obtained.

4. Conclusions

The levels of pathogenic bacteria present in animal faeces have been identified as a critical input in many farm-to-fork risk assessments. Such loading can be difficult to characterise; however, Bayesian inference allows for collating several data sources to provide a likely estimate of disease *P*. In conjunction with typical loading values in the manure of infected animals, such data can yield an estimate for bacterial loading in FYM&S. This methodology was used for a suite of target pathogens in determining the *P* of a range of animal diseases in cattle, pigs, and sheep. There were data gaps for some target pathogens in pig and sheep. Further research in this area will improve the estimation and reduce the confidence interval. This study estimated the mean *P* (P_{mean}) of *Cryptosporidium* spp., MAP., *Salmonella* spp., *Clostridium* spp., and *Campylobacter* spp. in cattle as 25.93%, 15.68%, 2.95%, 5.88%, and 8.80%, respectively. The P_{mean} of *Cryptosporidium* spp., *Salmonella* spp., *Clostridium* spp., and *E. coli* in pig was evaluated as 0.96%, 2.98%, 0.51%, and 7.42%, respectively. The P_{mean} of *Listeria monocytogenes* and *Clostridium* spp. in sheep was 6.70% and 7.94%, respectively. Initial estimates for *Cryptosporidium* spp., MAP., *Salmonella* spp., *Clostridium* spp., and *Campylobacter* spp. in cattle faeces were derived with an average concentration of 2.69, 4.38, 4.24, 3.46, and 3.84 $\log_{10}$ MPN g $^{-1}$, respectively. Similarly, the average $C_{initial}$ of *Cryptosporidium* spp., *Salmonella* spp., *Clostridium* spp., and *E. coli* in pig slurry was estimated as 1.27, 3.12, 3.02, and 4.48 $\log_{10}$ MPN g $^{-1}$, respectively. It was only possible to evaluate the average $C_{initial}$ of *Listeria monocytogenes* in sheep manure as 1.86 $\log_{10}$ MPN g $^{-1}$. This methodology allows the synthesis of multiple data sets to get an overarching approximation of the likely levels of pathogens in FYM&S and facilitates more accurate risk estimates.

CRediT authorship contribution statement

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

Declaration of competing interest

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

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