Yersinia enterocolitica in wild and peridomestic rodents within Great Britain, a prevalence study

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Impacts:

- The first documented account of positive culture of *Yersinia enterocolitica* in six wildlife species in the United Kingdom, and entirely novel in two species.
- The prevalence of *Yersinia enterocolitica* within wildlife species in the United Kingdom is comparable to the documented prevalence in other countries.
- All *Yersinia enterocolitica* positive wildlife species, are positive with the non-pathogenic biotype, supporting the EFSA's theory that biotype 1A is commonly found at low prevalence within wildlife.

Abstract

Yersinia enterocolitica is a human pathogen transmitted via the faecal-oral route among animals and humans and is a major food-borne public health hazard. This study explores the role of *Yersinia enterocolitica* transmission at the livestock-wildlife interface and investigates the potential role wild and peridomestic rodents play as a source of this zoonotic pathogen. The total of 342 faecal samples collected from the seven rodent species and one insectivore was examined using an optimised protocol to culture and identify *Y. enterocolitica*. Positive samples were also sero-biotyped for grouping and determination of sample pathogenicity. Wildlife species sampled in this study were separated into two sample groups: randomly sampled (brown rats, house mice, wood-mice, bank voles, field voles and the common shrew), as well as targeted sampling (red and grey squirrels). The overall prevalence of *Y. enterocolitica* in the randomly sampled population was 3.73%. Brown rats were chosen as sentinel species and tested to determine if location (pig farm vs non-pig farm) was a significant factor affecting *Y. enterocolitica* prevalence. In this study, location was not significant. All positive samples were found to be of biotype 1A, deemed non-pathogenic. Three of the samples were serotype 09, six were serotype 27, and five had an unidentifiable serotype. This study represents the first time *Y. enterocolitica* has been identified in these species of wildlife within mainland Britain. In addition, this study's findings are entirely novel and overall with regards to field voles and common shrews. However, the role of wild and peridomestic rodents in the transmission of pathogenic *Y. enterocolitica* remains unknown, as this study was unable to detect the presence of pathogenic *Y. enterocolitica* strains in these species.

1 Introduction

The UK Food Standards Agency (FSA) estimates that foodborne disease cost the UK economy £1.5 billion pounds annually (FSA, 2015). In 2018, yersiniosis was the fourth most reported zoonotic disease within the European Union (EU) with 6,699 confirmed cases caused by *Yersinia enterocolitica (Y. enterocolitica)*, with the trend of human yersiniosis cases remaining stable between 2014-2018 (EFSA, 2019). As such, the European Food Safety Authority (EFSA) considers *Y. enterocolitica* as one of the priority foodborne hazards associated with pork consumption requiring control in the pork chain (Blagojevic & Antic, 2014; EFSA, 2011). The agent is transmitted via the faecal-oral route among animals and humans, and has been identified as one of the four main food-borne public health hazards associated with domestic swine production, alongside *Salmonella*, *Trichinella* and *Toxoplasma* (EFSA, 2011). Furthermore, EFSA concludes that the most appropriate indicator of *Y. enterocolitica* pathogenicity is the biotype rather than serotype, as several serotypes are known to be both pathogenic and non-pathogenic (EFSA, 2011). However, serotype is useful to further classify strains based upon significant serotype-specific cell binding and cell entry characteristics (Schaake et al., 2013). Biotype is determined by the presence of virulence factors associated with the 0-antigen group; these include a heat-stable endotoxin and a virulence plasmid (Harnett et al., 1996). Clinically, the pathogenic genes which code for virulence factors, namely *VirF* (regulatory gene), *ail* (adhesion and invasion) and *Yst* (enterotoxin) are able to be detected via a Polymerase Chain Reaction (PCR) and are useful for organism identification and disease diagnosis (Bancerz-Kisiel et al., 2018). However, pathogenic genes are not solely found within pathogenic strains of *Y. enterocolitica* (Harnett et al., 1996).

Bioserotypes of enteropathogenic *Yersinia* are linked to different geographical areas (Bottone, 1999; Fukushima et al., 2001). Within Europe, Bioserotype 4/O:3 and Bioserotype 2/O:9 are frequently connected with enteric zoonotic infections, and are becoming increasingly prevalent in North America, where 1B/O:8 and 2/O:8 have historically been the predominant bioserotypes (EFSA, 2011; Jones et al., 2003; Shayegani et al., 1983; Tauxe, 2002). Within Europe, there appears to be a geographical bias regarding *Y. enterocolitica's* prevalence. The majority of studies have largely documented increased prevalence in Northern European Countries; Norway, Finland, Sweden and Denmark (Martínez, 2010;

Nesbakken et al., 2003; Niskanen et al., 2003), especially with regards to wildlife (Kapperud & Rosef, 1983). This is further reinforced by Fredriksson-Ahomaa et al. (2000), who discovered that Northern, compared to Southern European countries such as Italy and Greece (Bonardi et al., 2003), have a higher prevalence of *Y. enterocolitica*.

Domestic pigs (*Sus scrofa domesticus*) have previously been identified as the primary reservoir of foodborne human pathogenic *Y. enterocolitica* strains (Powell et al., 2016; Wauters, 1979), for which they are asymptomatic carriers. The mode of transmission between pigs and people is predominantly foodborne; with most cases occurring due to consumption of undercooked pork contaminated with *Yersinia* from pig faeces or tonsils (Grahek-Ogden et al., 2007). To combat this, reducing the occurrence of *Y. enterocolitica* at the farm level is essential (Virtanen et al., 2012) and to achieve this goal, a greater understanding is needed of possible sources of contamination at the farm level (Tauxe et al., 1987).

The wildlife-livestock interface can be defined as the physical space where wildlife and livestock meet, interact and share resources, pasture, water, etc (Bengis et al., 2002). Murphy (2018) documented that farm environments have become anthropogenic habitats which a wide variety of rodent species exploit, and British pig farms are environments which support large rodent populations and have the potential for zoonotic pathogen transmission. This increased risk of interspecies disease transmission, involving rodents on farms, is recognised by the FSA who enforce “controlled housing schemes” as a method of transmission risk reduction (Franssen et al., 2018). As such, a large emphasis is placed on suitable pest-control programmes designed to target rodents. This is especially important for free-range pig units, which do not meet the criteria for controlled housing schemes as documented by the European Commission (EC 2015/1375) (2015).

Currently, no wildlife reservoir of pathogenic *Y. enterocolitica* has been identified (Backhans et al., 2011), however rodents are known to be infected with the pathogenic 4/O:3 strain of the agent, *e.g.* mice (Schaake et al., 2013), and black and brown rats (Kaneko et al., 1978). Nevertheless, these findings are rare, and the majority of studies confirm Kapperud (1975) who found non-pathogenic *Y. enterocolitica* in 8% of wild rodents in Scandinavia. Moreover, Backhans et al. (2011) sampled brown rats and house mice from pig farms in Sweden; their results demonstrated only rodents caught on pig farms tested positive for the pathogen *Y. enterocolitica* bioserotype 4/O:3. This suggests that pigs are the source of infection for other

animal species, as well as being a reservoir of pathogenic *Y. enterocolitica* in people. These discrepancies necessitate the determination of rodents species' role as a potential reservoir of pathogenic *Y. enterocolitica* (Backhans & Fellström, 2012).

As no research has examined the role wild or peridomestic rodents play as potential reservoirs of pathogenic *Y. enterocolitica* within the United Kingdom (UK), this pilot study was designed to discover the prevalence of *Y. enterocolitica* within wild and peridomestic rodents from various locations across the UK, including pig farms, non-pig farms and wild areas.

2. Materials and Methods

The ethical approval for this research project and its methods was granted by the University of Liverpool and the University of Edinburgh. Previous research performed by Murphy (2018), created a biobank of wildlife samples which were utilised in this study. A wide range of wild and peridomestic rodent species (n=7), and one insectivore (n=1) were collected from 22 different locations, categorised into pig farm, or any other location (non-pig farm) (Table 1)(Table 2) (Figure 1). Rodents were trapped from various locations including multiple pig farms, to reflect the diverse environments that peri-domestic and wild rodents inhabit. Species samples were frozen at -20°C on-site in a mobile freezer, before being transported to the University of Liverpool's Leahurst campus for processing and long-term storage at -80°C in a biobank of rodent tissue.

Table 1

Table 2

Figure 1

Gastrointestinal tract samples of 342 rodents were collected from the -80°C freezer the morning before extraction and left for 24 hours to defrost at 7°C in a refrigerator. Approximately one gram of faecal contents was collected from the gastrointestinal tract of

each rodent sample and added into nine ml of Irgasan Ticarcillin Chlorate (ITC) enrichment broth (Momtaz et al., 2013). The broths were incubated for 24 hours at 30°C and subcultured onto Cefsulodin Irgasan Novobiocin (CIN) agar and incubated for 36 hours at 30°C (Fukushima, 1987).

Yersinia species identification was undertaken by examining the CIN agar plates under a light microscope, one colony from each species that met the morphological criteria (Devenish & Schiemann, 1981) was utilised in further steps. Following culture on CIN agar, one colony of suspected *Y. enterocolitica* from morphological diagnosis was subcultured onto Plate Count Agar (PCA) and incubated at 30°C for 24 hours (Van Damme et al., 2010). In addition, one colony of the bacterium was placed onto a urea slope (Thermo Fisher, Loughborough) and incubated at 35°C for 24 hours (Laukkanen et al., 2010). Comparison of microscope results and urea slope results was used as a screening test, and positive results were utilised in further subsequent analysis.

2.1. PCR

After 24 hours, from pure growth of *Y. enterocolitica* on the PCA, five colonies were added to a sterile microcentrifuge tube containing 300 µl of nuclease free water (Sigma Aldrich, Zurich Switzerland), the solution was then vortexed for 30 seconds and heated at 95°C for 10 minutes to extract DNA for use in polymerase chain reactions (PCR) (Table 3).

Table 3

PCR conditions for detecting biotypes and pathogenicity were as follows. In a total volume of 25 µl containing 1 x FIREPol® Master Mix Ready to Load (Solis Biodyne, Estonia), each primer combination at 500 nM each, 1 µl of the boiled prep was used as the template and the total volume was made up with nuclease free water (Sigma Aldrich, Zurich Switzerland). A negative control with each of the reaction components, except sample DNA, using DNA free H_2O water (Sigma Alderich, Dorset, UK) was utilised. As a positive control, 1 ng of purified genomic DNA were provided by Public Health England and were used in an early *Y.*

enterocolitica project conducted at the University of Liverpool. These samples were positive for all pathogenic genes and each serotype of interest was present as well.

Amplification was performed in a Primus 96 Plus thermal cycler (MWG Biotech Inc, North Carolina, USA). Cycling conditions were as follows; 94°C for 10 min, followed by 30 or 35 (pathogenic or serotype PCR respectively) cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. A final extension was performed at 72°C for 7 min to complete the synthesis of all strands.

A volume of 8 µl aliquots of the PCR amplified product were analysed by agarose gel electrophoresis on a 2% agarose Peg green gel for 70 minutes. The PCR products were visualised under UV light. Molecular size markers included in all gels were the '100 bp DNA Ladder Ready to Load' (Solis Biodyne, Estonia).

2.2. Other confirmatory tests

An 'Analytical Profile Index' (API), API® 20E (bioMérieux, Marcy-l'Étoile, France), was undertaken on all isolated *Y. enterocolitica* cultures as a method of confirming growth of bacterium. To complete an API, 5 ml of sterile saline and one colony of nutrient agar grown suspected *Y. enterocolitica*, was placed into a sterile Bijou and vortexed for 10 seconds to make a suspension. The results generated were entered onto the APIWEB™ internet database which provided bacterial identification of the cultured organism.

MALDI-TOF MS was undertaken in-house by the University of Liverpool's Veterinary Microbiology Diagnostic Laboratory. The Bruker system (MALDI Biotyper (MBT), Bruker Daltonics GmbH & Co. KG.) database and the MBT Compass Library was accessed June 26th 2019. Samples of pure culture grown on nutrient agar were provided to the diagnostic laboratory for MALDI-TOF MS analysis.

Final confirmatory diagnosis of bacterial identification was undertaken via 16s rRNA sequencing using the methodology described by Wannet et al. (2001). Primers specific for *Y. enterocolitica* 16S rRNA as described by Neubauer et al. (2000) were ordered from Eurofins Genomics (Luxembourg, Luxembourg), Y.E 1 (AAT ACC GCA TAA CGT CTT CG) and Y.E 2 (CTT CTT CTG CGA GTA ACG TC). PCR conditions were as follows, in a total volume of 25 µl containing 1 x PCR reaction buffer (with 1.5 mM $MgCl_2$, ThermoFisher, DE, USA), 200 µM dNTPs, primers Y.E 1 & 2 were at a final concentration of 80 nM, 0.5 U

SuperTaq™ DNA polymerase (ThermoFisher, DE, USA), 1 µl of the boiled prep was used as the template and the total volume was made up with nuclease free water (Sigma Aldrich, Zurich Switzerland). Amplification was performed in a Primus 96 Plus thermal cycler (MWG Biotech Inc, North Carolina, USA). Cycling conditions were as follows; 94°C for 5 min, followed by 36 cycles of 94°C for 45 s, 62°C for 45 s, and 72°C for 45 s. A final extension was performed at 72°C for 7 min to complete the synthesis of all strands. The PCR products were visualized on 1.5% agarose gels stained with ethidium bromide to confirm single amplicons were produced.

Appropriate single amplicon PCR products were submitted to Source Bioscience (Nottingham, UK) for bi-directional Sanger sequencing, where a commercial clean up protocol was used. Ten out of the sixteen samples submitted returned with successful *Y. enterocolitica* identification. The 16S rRNA sequencing was repeated on the six failed samples, two of which returned positive results.

DNA sequences from all presumptive positive samples were trimmed and aligned to the reference sequence accession numbers downloaded from NCBI Genbank (NCBI, 2016) and aligned using Geneious (2021) (10.2.6).

2.3. Bioserotyping

In addition to the serotype PCR (Table 3), which was targeting serotypes 03 and 09, additional methods to determine pathogenicity of the *Y. enterocolitica* samples were undertaken. Three other pathogenic serotypes were tested for: 05, 08 and 27, using a slide agglutination test (Sifin Diagnostics, GMBH, Berlin, Germany). Slide agglutination was undertaken via the addition of antibody to the specific serum, to a loop of pure growth *Y. enterocolitica* (from the nutrient agar plate) and by mixing vigorously on a microscope slide. A negative reaction occurred if the reaction stayed milky white and homogenous, whilst a positive reaction was noted if samples agglutinated into visible clumps.

Biotyping was undertaken following the prescribed methodology of BS EN ISO 10273:2003, based on the metabolism of trehalose, xylose, bile esculin and indole. A pure growth colony was taken from the nutrient agar plate and plated onto phenol red medium. Commercially available trehalose and xylose sugar discs (Sigma Alderich, Dorset, UK) were placed opposite to each other at the widest part of the agar plate and left to incubate at 30ºC for 24

hours. Positive results indicating the bacteria was able to breakdown the sugar resulted in a vibrant colour change, negative results showed no colour change.

Separate nutrient agar plates were used to identify the isolates' ability to break down bile aesculin. A single colony of pure growth was subcultured to a new nutrient agar plate and a bile esculin disk (Sigma Alderich, Dorset, UK) was placed onto the new plate. These plates were incubated at 30ºC for 24 hours, a positive result was a colour change from clear to black, in the agar indicating the bacteria's ability to metabolise bile esculin, negative results showed no colour change. Indole reactions were recorded from the use of API® E 20.

2.4. Data analysis

Estimation of overall and species-specific prevalence was performed using R (R Core Team) version 4.0.5 (2021-03-31). All scripts used for this analysis are available in the Supplementary Material. To calculate the overall prevalence, the samples were split into two populations: rodents who were randomly sampled (rats, house mice, wood-mice, bank voles and field voles), and rodents who were targeted (red and grey squirrels). The one common shrew, who was an accidental catch, and who was positive for *Y. enterocolitica*, was removed from overall prevalence calculations.

The overall unadjusted prevalence of the randomly selected population was estimated using a generalized linear model with only an intercept term, using the glm function. The predicted probability of *Y. enterocolitica* presence was reported as the unadjusted overall prevalence, and the corresponding standard error used to construct a Wald 95% confidence interval.

The field voles appeared to form a 'hot spot' as the majority of positive field vole samples were found within a national park in North Wales. Thus, the overall prevalence was adjusted to account for the effect of UK counties from which the observations were reported. To this effect, a generalised linear mixed-effects model was constructed, using the glmer function from the lme4 R package (Bates et al., 2015). The model included an intercept term and the County variable as a random effect. The overall adjusted prevalence and corresponding 95% confidence interval were computed using the predictInterval function from the merTools R package (Knowles et al., 2016), setting the number of simulations to 10,000.

Each individual species prevalence was also calculated, by adding to the previous model (for adjusted prevalence) the species as a fixed effect term. The merTools package was also used to obtain the estimated species prevalence and 95% confidence intervals.

To test whether rodent species present an increased prevalence of *Y. enterocolitica* on pig farms compared to non-pig farms, the observations corresponding to rats were used. This species was chosen due to there being marginally more *Y. enterocolitica* positive samples, and because this species is commonly found on pig farms (Murphy, 2018). A generalised linear model was constructed on the rats observations only, with only an intercept, using the glm function. Next, the pig farm indicator variable was added to the model. A likelihood ratio test was performed to assess whether the inclusion of the pig farm indication variable significantly improved the model. In addition, a Fisher's exact test was performed for each species separately (i.e. rats and wood mice), to assess whether the type of farm and presence of *Y. enterocolitica* were independent.

The unadjusted prevalence for each targeted rodent species was calculated with a generalized linear model with an intercept term and a species term, using the glm function. The predicted probability of *Y. enterocolitica* presence for each species was reported, and the corresponding standard errors were used to construct a Wald 95% confidence interval for each species. As both targeted species were found in the same county (Merseyside), there was no need to account for the county effect.

3. Results

3.1. *Yersinia enterocolitica* identification

Out of the total 342 faecal samples collected from the seven rodent species and one insectivore, fourteen samples from five species were confirmed positive for *Yersinia enterocolitica* via MALDI-TOF, with eleven of these samples being additionally confirmed positive by 16s rRNA sequencing (Table 4).

Table 4

As part of the biobank, metadata was available for all species sampled in this study. Sex and age were variables which were originally believed to be worth investigating in relation to *Y. enterocolitica* prevalence. However, by conducting Fisher Exact tests on both age and sex across all rodent species used in this study, it was shown that sex (0.7244) was not a significant factor in relation to prevalence of *Y. enterocolitica*, and as such further analysis regarding this factor was not undertaken. Age was determined to be a significant factor (0.0011) regarding prevalence of *Y. enterocolitica* when all 7 species were analysed as a collective. However, on an individual species level, age was not significant in any species and as such was not used as factors during the prevalence calculations.

3.2. *Yersinia enterocolitica* prevalence

The overall unadjusted prevalence of *Y. enterocolitica* in the randomly sampled population was 3.73%, (95% CI: 2.08%, 6.61%). The overall prevalence of *Y. enterocolitica* in the randomly sampled population, adjusting for County effect, is 2.38%, (95% CI: 0.47%, 11.54%). The estimated prevalence of *Y. enterocolitica* per species, adjusting for the County effect is presented in Table 5.

Table 5

3.3. Farm type effect on *Yersinia enterocolitica*

To analyse the effect of farm type on *Y. enterocolitica* prevalence, two designed models were created (see supplementary) using rats as sentinels of *Y. enterocolitica* prevalence on pig farms. The likelihood ratio test showed that the farm type (pig.farm variable) has an effect on the prevalence of *Y. enterocolitica* in rats (p-value of 0.047). Interestingly, more *Yersinia* positive samples were found in ‘non-pig farm’ areas compared to on pig-farms, with the proportion of *Y. enterocolitica* positive rat samples being 0.03 on pig farms, compared to 0.08 on non-pig farms.

Fisher’s exact tests were attempted for the sentinel species to examine the association between pig farm vs non-pig farm prevalence. The result (0.2416) was not significant. A further Fisher’s exact test was attempted on all 14 *Y. enterocolitica* positive samples to

determine if pig-farm was significant on the positive population as a whole. In this instance the result was significant ($p \leq 0.001$), indicating an association between pig-farm and non-pig farm *Y. enterocolitica* prevalence does exist.

3.4 *Yersinia enterocolitica* pathogenicity

In this study serotyping was undertaken by a mixture of PCR and commercial serum agglutination. Three of the samples were serotype 09 (R13, R34 & WM24), six were serotype 27 (BV 16, FV1, FV4, FV7, FV9 and FV10), and five had an unidentifiable serotype (R1, R10, WM27, GS35 and SHW1) (Table 6). Following identification of the serotype, biotyping was undertaken. All 16 *Yersinia enterocolitica* positive samples were found to be of biotype 1A, deemed non-pathogenic by the International Standard's Organisation (ISO) (Table 6).

Table 6

4. Discussion

Rodents are an order of crucially important animals in terms of public health. This is due to rodents adaptability to thrive in both wild and anthropogenic environments, resulting in the transfer of pathogens at the livestock-wildlife (Young et al., 2014) and human-animal interfaces (Siembieda et al., 2011). The aim of this pilot project was to investigate the prevalence of the zoonotic foodborne disease *Y. enterocolitica* (Naktin & Beavis, 1999), within the sampled population of British wild and peridomestic rodents. The samples were collected from a wide range of locations throughout the UK, including pig farms, rural areas, and urban environments.

The first step in this study involved optimising a protocol for the culture and extraction of *Y. enterocolitica.* All isolates of presumptive *Y. enterocolitica* were confirmed as the agent of interest against commercially available standard tests (MALDI-ToF, API and 16S RNA Sequencing). In this study *Y. enterocolitica* was successfully identified in brown rats, wood mice, bank voles, field voles, grey squirrels, and common shrews. This study represents the first time *Y. enterocolitica* has been identified in these species of wildlife within mainland

Britain. In addition, this study's findings are entirely novel with regards to field voles and common shrews. Owing to this study's success with the cultivation, isolation, and confirmation of *Y. enterocolitica* culture methodology, the 'Diagnostic Service' of the Veterinary Science Department at the University of Liverpool is now using the protocol used in this study to culture *Y. enterocolitica* from a range of biological sources provided by customers requiring case diagnosis.

The overall prevalence of *Y. enterocolitica* in the sampled population, adjusting for County effect, is 2.38% (Table 5). Observational biases which had been made regarding sex and age were found to be statistically non-significant. This could largely be due to the low number of positive samples per species: brown rats (4/70), house mouse (0/86), wood mouse (2/75), bank voles (1/47), field voles (5/17), red squirrels (0/10), grey squirrels (1/36) and common shrew (1/1). These results highlight one of the problems in conducting wildlife surveillance studies; that a large sample size is not guaranteed. Furthermore, it was also not possible to determine the required sample size for this study as there was no prevalence data available for *Y. enterocolitica* in wildlife within the UK. As such, it was not possible to conduct a power calculation.

One species was chosen as a sentinel to investigate a possible association of prevalence and pig farms. Rats were chosen owing to their comparatively higher number of *Y. enterocolitica* positive samples, and an increased number of species trapped on pig farms (33/70). Originally, a generalized linear model in R was attempted. The likelihood ratio test showed that the farm type (pig.farm variable) has an effect on the prevalence of *Y. enterocolitica* in rats (p-value of 0.047), however the Fisher's exact test result (0.2416) was not significant. Despite the rats having a comparatively higher number of *Y. enterocolitica* positive samples compared to other species in this study, caught at mixture of locations, the sample number was still too small.

The final Fishers' exact test was undertaken on the total positive rodent population. Despite the Fisher's exact results being significant ($p \leq 0.001$), the result disagrees with these studies aims, as more *Yersinia* positive samples were found in 'non-pig farm' areas. This finding supports Pocock et al. (2001), who believed that *Y. enterocolitica* is commensal in rodents. The Fisher's exact test was undertaken out of curiosity to help decern if the studies aims were correct, however its results do not take into account clustering hotspots such as the wild

population of field voles in Snowdonia national park (Murphy, 2018). Furthermore, it does not distinguish between the randomly sampled, where each species has an equal chance of being selected, and the targeted rodent populations. Its findings are therefore not reliable.

In this experiment all samples which were deemed potentially *Y. enterocolitica* positive from morphometric diagnosis progressed to the next stage of identification and were analysed via PCR to identify the pathogenic genes *ail*, *yst* and *virF* (Harnett et al., 1996). Three samples, one field vole (FV1), one grey squirrel (GS35) and one wood mouse (WM24), were confirmed *Y. enterocolitica* positive by diagnostic testing but did not produce any amplicons to any of the three aforementioned genes via PCR. Despite the DNA being successfully amplified for the 16s RCR. This is not unexpected, as these genes are most often found in pathogenic strains of *Y. enterocolitica* (Floccari et al., 2004). However, despite all samples being confirmed as non-pathogenic biotype 1A present, the remaining samples were positive for one or multiple pathogenic genes. Natural selection and mutations of bacteria in hosts, vectors and reservoirs are an essential component of the incidence and prevalence of bacterial diseases and these processes lead to the emergence of innovative antigenic and pathogenic features (Han et al., 2015). Platt-Samoraj et al. (2020) discovered five *Y. enterocolitica* isolates that despite being classified as biotype 1A also harboured the y*stB* as well as *ail gene*, an important marker of pathogenicity. Although, virulence genes are known to be present in biotype 1A such as *yst* (Thoerner et al., 2003) and *ail* (Sihvonen et al., 2011), these are rare findings, and as such the results of this study, similar to those of Platt-Samoraj et al., (2021) are unexpected and require further investigation and research to explore alternative explanations for these findings. Nevertheless, Thoerner et al. (2003) describes how it is not possible to distinguish between pathogenic and apathogenic isolates within this biotype based on the virulence gene detection alone, and instead requires biotyping to truly identify a pathogenic strain, a sentiment which is reinforced by this study's and multiple other studies results (Platt-Samoraj et al., 2021; Sihvonen et al., 2011)

With regards to the *virF* gene, this study isolated *virF* in seven samples: three rats (R10, R13 and R34), two woodmice (WM27 and WM63) and two field voles (FV7 and FV10). This gene is challenging to isolate due to the heterogeneity within the bacterial population with regards to the presence of the virulence plasmid, meaning it could be present in more samples but not detected (Thoerner et al., 2003). However, what is surprising is that *virF* has only been previously identified in pathogenic biotypes. The rationale behind these results is unclear, potentially some form of gene transference could have occurred (Brem et al., 2001),

as these samples were all collected in a location containing *virF* positive and negative *Y. enterocolitica.* This is an area which needs to be explored further in future studies, to determine the full functionality of the *Y. enterocolitica* pathogenic genes and the complexity of the system, and discover if, similar to *ail* and *yst, virF* is identified in other non-pathogenic samples.

Two serotypes, out of five which were tested for, were confirmed in this study: 09, in two brown rats (R13 and R34) and one wood mouse (WM24), and serotype 27, in all positive bank voles (BV16) and field voles (FV1, FV4, FV7, FV9 and FV10). The remaining five samples had an unidentifiable serotype. Whilst this is not unprecedented as there are over 50 serotypes recorded (Miller et al., 1989), and testing all known serotypes was not financially viable nor warranted in this study, the results of this study are still unexpected as the common serotypes 03, 05 and 08 (Liang et al., 2015; McNally et al., 2004; Shayegani et al., 1986), found in human, domestic animals and wildlife were tested for; whilst 09 is rarer in wild animals (Kaneko & Hashimoto, 1981), and 27 is most commonly found in domestic pigs (Kotula & Sharar, 1993). It is therefore especially interesting that a cluster of serotype 27 positive field voles were found within Snowdonia National Park. In future work, biotyping samples for pathogenicity will be prioritised and samples will be tested for a greater range of common serotypes, in order of likeliness based on published data.

This study's results are in line with published prevalence data of similar studies examining rodents and *Y. enterocolitica* in other countries. Liang et al. (2015) recorded a prevalence of 3.40% non-pathogenic *Y. enterocolitica* in rodents and 6.76% in Plateau pica (*Ochotona curzoniae*) within China, whilst Shayegani et al. (1986) recorded an overall prevalence of 7% in the various wildlife species samples of various serotypes. The majority of studies, including this one, support the EFSA's belief that biotype 1A is commonly found at low prevalence within wildlife. The discovery of a cluster of serotype 27 *Y. enterocolitica* positive field voles in Snowdonia is interesting and worth investigating further to decipher if this cluster does reflect an abnormal high prevalence of *Y. enterocolitica* in this population. Furthermore, it is worth discovering if there are any pathogenic strains prevalent within this population, as it is known within domestic pigs that both pathogenic strains and non-pathogenic strains are found within a population (McNally et al., 2004). Moreover, it is important to explore whether other species within this area are positive for *Y. enterocolitica*.

In this study, no red squirrels were positive for *Y. enterocolitica*, however one grey squirrel was. The observed decreased prevalence is likely to be due to the location in which these species were caught; all within a forest park surrounded by urban environments and the coast. Unfortunately, this study has not provided an insight as to whether grey squirrels can transmit *Y. enterocolitica* to red squirrels, but it has confirmed for the first time that grey squirrels within the UK do harbour *Y. enterocolitica*, similar to Shayegani et al. (1986) who discovered the agent in grey squirrels in North America. This is an area which requires further research as it is known that rodents can be infected by pathogenic *Y. enterocolitica*, as Backhans et al. (2011) documented a prevalence of 5% of pathogenic biotypes in rats in Sweden. In their study, all positive pathogenic biotypes were isolated from rats found around pig farms, suggesting that pigs could be passing pathogenic *Y. enterocolitica* to rodents. Nevertheless, this shows that pathogenic *Y. enterocolitica* can cross the species barrier outside of the documented pig to human direction. This could have far-reaching public health implications if the rodents are able to transmit the disease to other peri-domestic or domestic species once infected. Further work is necessary to determine whether this transmission occurs.

The success of this study is apparent, as this was the first time *Y. enterocolitica* has been identified in these species of wildlife within the United Kingdom, and entirely novel with regards to the identification of *Y. enterocolitica in* field voles and common shrews. Moreover, this study laid the groundwork for further research regarding *Y. enterocolitica* disease transmission at the livestock-wildlife interface and to further explore the relationship between non-pathogenic and pathogenic strains of *Y. enterocolitica* within wildlife in the UK.

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6. Ethical Statement

The ethical approval for this research project was granted by the University of Liverpool (VREC267ab (2nd amendment)). Approval from the University of Edinburgh was granted as a stand-alone research project utilising the biobank of rodent samples at the University of Liverpool.

7. Conflict of Interest

There is no conflict of interest to declare. This study was completed in partial fulfilment of the Degree of Masters of Veterinary Science at the University of Edinburgh Royal (Dick) School of Veterinary Studies.

8. Tables

Table 1 The wildlife species, total number sampled and categorised trap location (pig farm or non-pig farm, rural or urban) adapted from Murphy (2018)

Species	Farm type		Rural or urban		Total sample number
	Pig farm sample number	Non-pig farm sample number	Rural	Urban	
Brown rats (*R. norvegicus*)	33	37	38	32	70
House mice (*Mus musculus*)	84	2	86	0	86
Wood mice (*Apodemus sylvaticus*)	41	34	73	2	75
Bank voles (*Myodes glareolus*)	42	5	47	0	47
Field voles (*Microtus agrestis*)	1	16	17	0	17
Red squirrels (*Sciurus vulgaris*)	10	0	10	0	10
Grey squirrels (*Sciurus carolinensis*)	36	0	4	32	36

Common shrew (*Sorex araneus*)	1	0	1	0	1
Total	248	94	276	66	342

Table 2 The location and description of collection site, species collected, and number utilised in this study adapted from Murphy (2018).

Table 2 The location and description of collection site, species collected, and number utilised in this study.

Code	Location	Species Collected	Map Number	Site Description
Pig Farm 1	Yorkshire	Brown rat (n=13), house mouse (n=1), wood mouse (n=1), bank vole (n=8)	A	Indoor pig unit
Pig Farm 2	Cheshire	Brown rat (n=7)	B	Outdoor pig unit
Pig Farm 3	Northumberland	House mouse (n=37)	C	Indoor pig unit
Pig Farm 4	Kingston Upon Hull	Brown Rat (n=1), house mouse (n=4), Bank voles (n=1)	D	Indoor pig unit and chicken farm
Pig Farm 5	Northumberland	House mouse (n=6), Bank voles (n=2)	E	Outdoor pig unit (rare breed)
Pig Farm 6	Yorkshire	Brown Rat (n=1), House mouse (n=3), Wood mouse (n=9), Bank voles (n=14).	F	Indoor and outdoor pig unit.
Pig Farm 7	Edinburgh	House mouse (n=6), wood mouse (n=9)	G	Indoor pig unit.
Pig Farm 8	Yorkshire	House mouse (n=4), Wood voles (n=1), Bank voles (n=9)	H	Outdoor pig unit.
Pig Farm 9	Yorkshire	Brown rat (n=11), Wood mouse (n=3), Bank voles (n=6)	I	Outdoor pig unit.
Pig Farm 10	Northumberland	Wood mouse (n=19), Bank voles (n=2)	J	Outdoor pig unit.
Pig Farm 11	Shrewsbury	House mouse (n=2)	K	Indoor pig unit
Pig Farm 12	Yorkshire	House mouse (n=16)	L	Indoor pig unit
Farm 1	Cheshire	Brown rat (n=5), Bank vole (n=1), Wood mouse (n=18)	M	Indoor dairy unit
Farm 2	Derbyshire	Brown rat (n=8)	N	Outdoor beef unit
Farm 3	Cheshire	Field vole (n=2), Bank vole (n=3)	O	Outdoor beef unit
Rural 1	Llyn Cowyld	Field vole (n=14), Bank vole (n=1), Brown Rat (n=2)	P	Reservoir
Rural 2	Ruthin	Brown Rat (n=4)	Q	Small holding

Urban 1	Liverpool	Brown rat (n=4)	R	Commercial premise
Urban 2	Cheshire	Brown rat (n=11)	S	Residential premise
Forest 1	Formby	Grey squirrel (n=36), Red squirrel (n=10)	U	Range of locations

Table 3 Target genes for serotyping and pathogenicity testing, the forward and reverse primer sequences, and amplified products (in basepairs).

Target Gene	Test	Size of amplified product (bp)	Sequence	Reference
ail	pathogenicity	356	F: TGGTTATGCGCAAAGCCATGT	Harnett et al. (1996)
			R: TGGAAGTGGGTTGAATTGCA	
yst	pathogenicity	134	F: GTCTTCATTTGGAGGATTCGGC	Harnett et al. (1996)
			R: AATCACTACTGACTTCGGCTGG	
virF	pathogenicity	231	F: GCTTTTGCTTGCCTTTAGCTCG	Harnett et al. (1996)
			R: AGAATACGTCGCTCGCTTATCC	
rfbC	serotyping	405	F: CGCATCTGGGACACTAATTCG	Weynants et al. (1996)
			R: CCACGAATTCCATCAAAACCACC	
per	serotyping	181	F: TGTGCTGAAGCTTTTGGATCT	Jacobsen et al. (2005)
			R: GAGGCCGATACACCTTGATT	

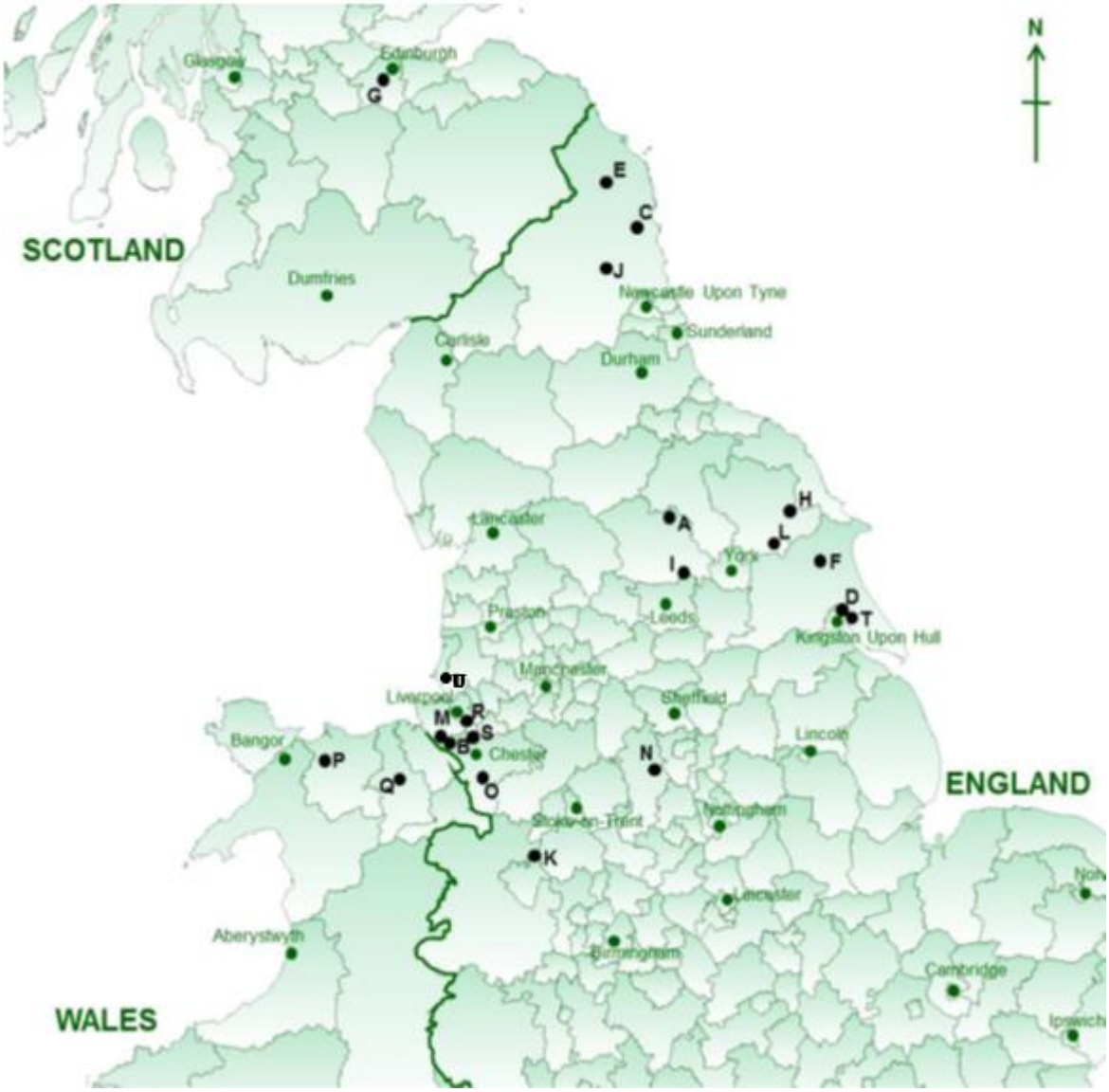
Table 5: The overall prevalence of *Yersinia enterocolitica* of each species sampled, (with 95% confidence interval), the population the sample belonged to, and whether the prevalence was adjusted for 'County' or not.

Species	Overall Yersinia Prevalence %	Lower confidence interval %	Upper confidence interval %	Adjusted for County
Rat	3.87	1.05	13.15	Yes
Wood Mouse	2.31	0.50	10.17	Yes
Bank Vole	2.61	0.31	18.98	Yes

Field Vole	29.62	10.34	60.22	Yes
Grey Squirrel	2.78	0.39	17.26	No
Red Squirrel	0	0	100.00	No
Common Shrew	100	0	0	No
Total	3.73	2.08	6.61	No
Total Adjusted	2.38	0.47	11.54	Yes

9. Figures

Figure 1 the location of trapping sites within England, Wales and Scotland were rodents were collected from Adapted from Murphy (2018).



10. Reference List

Backhans, A., & Fellström, C. (2012). Rodents on pig and chicken farms–a potential threat to human and animal health. *Infection Ecology & Epidemiology*, *2*(1), 17093.

Backhans, A., Fellström, C., & Lambertz, S. T. (2011). Occurrence of pathogenic Yersinia enterocolitica and Yersinia pseudotuberculosis in small wild rodents. *Epidemiology & Infection*, *139*(8), 1230-1238.

Bancerz-Kisiel, A., Pieczywek, M., Łada, P., & Szweda, W. (2018). The Most Important Virulence Markers of Yersinia enterocolitica and Their Role during Infection. *Genes*, *9*(5), 235. https://doi.org/10.3390/genes9050235

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. eprint. *arXiv preprint arXiv:1406.5823*, *51*.

Bengis, R., Kock, R., & Fischer, J. (2002). Infectious animal diseases: the wildlife/livestock interface. *Revue Scientifique et Technique-Office international des épizooties*, *21*(1), 53-66.

Blagojevic, B., & Antic, D. (2014). Assessment of potential contribution of official meat inspection and abattoir process hygiene to biological safety assurance of final beef and pork carcasses. *Food Control*, *36*(1), 174-182.

Bonardi, S., Brindani, F., Pizzin, G., Lucidi, L., D'incau, M., Liebana, E., & Morabito, S. (2003). Detection of Salmonella spp., Yersinia enterocolitica and verocytotoxin-producing Escherichia coli O157 in pigs at slaughter in Italy. *International journal of food microbiology*, *85*(1-2), 101-110.

Bottone, E. J. (1999). Yersinia enterocolitica: overview and epidemiologic correlates. *Microbes and infection*, *1*(4), 323-333.

Brem, D., Pelludat, C., Rakin, A., Jacobi, C. A., & Heesemann, J. (2001). Functional analysis of yersiniabactin transport genes of Yersinia enterocolitica. *Microbiology*, *147*(5), 1115-1127. https://doi.org/https://doi.org/10.1099/00221287-147-5-1115

Commission, E. (2015). Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for Trichinella in meat. *Off J Eur Union*, *58*, 7-34.

Devenish, J., & Schiemann, D. (1981). An abbreviated scheme for identification of Yersinia enterocolitica isolated from food enrichments on CIN (cefsulodin–irgasan–novobiocin) agar. *Canadian Journal of Microbiology*, *27*(9), 937-941.

EFSA, a. E. (2019). The European Union One Health 2018 Zoonoses Report. *EFSA Journal*, *17*(12), e05926.

EFSA, P. o. B. H. (2011). Scientific Opinion on the public health hazards to be covered by inspection of meat (swine). *EFSA Journal*, *9*(10), 2351.

Floccari, M. E., Neubauer, H. K., Gómez, S. M., Lodri, C., & Parada, J. L. (2004). Molecular characterization of Yersinia enterocolitica 1A strains isolated from Buenos Aires sewage water. In *The Genus Yersinia* (pp. 345-348). Springer.

Franssen, F., Takumi, K., van der Giessen, J., & Swart, A. (2018). Assessing the risk of human trichinellosis from pigs kept under controlled and non-controlled housing in Europe. *Food and Waterborne Parasitology*, *10*, 14-22. https://doi.org/https://doi.org/10.1016/j.fawpar.2018.04.003

Fredriksson-Ahomaa, M., Björkroth, J., Hielm, S., & Korkeala, H. (2000). Prevalence and characterization of pathogenic Yersinia enterocolitica in pig tonsils from different slaughterhouses. *Food Microbiology*, *17*(1), 93-101.

FSA. (2015). Foodborne Disease Stratergy 2010 - 2015. *1*(Food Standards Agency).

Fukushima, H. (1987). New selective agar medium for isolation of virulent Yersinia enterocolitica. *Journal of clinical microbiology*, *25*(6), 1068-1073.
Fukushima, H., Matsuda, Y., Seki, R., Tsubokura, M., Takeda, N., Shubin, F. N., Paik, I. K., & Zheng, X. B. (2001). Geographical Heterogeneity between Far Eastern and Western Countries in Prevalence of the Virulence Plasmid, the SuperantigenYersinia pseudotuberculosis-Derived Mitogen, and the High-Pathogenicity Island among Yersinia pseudotuberculosis Strains. *Journal of clinical microbiology*, *39*(10), 3541-3547.
Geneious. (2021). (10.2.6).
Grahek-Ogden, D., Schimmer, B., Cudjoe, K. S., Nygård, K., & Kapperud, G. (2007). Outbreak of Yersinia enterocolitica serogroup O: 9 infection and processed pork, Norway. *Emerging infectious diseases*, *13*(5), 754.
Han, B. A., Schmidt, J. P., Bowden, S. E., & Drake, J. M. (2015). Rodent reservoirs of future zoonotic diseases. *Proceedings of the National Academy of Sciences*, *112*(22), 7039-7044.
Harnett, N., Lin, Y., & Krishnan, C. (1996). Detection of pathogenic Yersinia enterocolitica using the multiplex polymerase chain reaction. *Epidemiology & Infection*, *117*(1), 59-67.
Jacobsen, N., Bogdanovich, T., Skurnik, M., Lübeck, P., Ahrens, P., & Hoorfar, J. (2005). A real-time PCR assay for the specific identification of serotype O: 9 of Yersinia enterocolitica. *Journal of microbiological methods*, *63*(2), 151-156.
Jones, T. F., Buckingham, S. C., Bopp, C. A., Ribot, E., & Schaffner, W. (2003). From pig to pacifier: chitterling-associated yersiniosis outbreak among black infants. *Emerging infectious diseases*, *9*(8), 1007.
Kaneko, K., Hamada, S., Kasai, Y., & Kato, E. (1978). Occurrence of Yersinia enterocolitica in house rats. *Appl. Environ. Microbiol.*, *36*(2), 314-318.
Kaneko, K., & Hashimoto, N. (1981). Occurrence of Yersinia enterocolitica in wild animals. *Applied and Environmental Microbiology*, *41*(3), 635-638. https://aem.asm.org/content/aem/41/3/635.full.pdf
Kapperud, G. (1975). Yersinia enterocolitica in small rodents from Norway, Sweden and Finland. *Acta Pathologica Microbiologica Scandinavica Section B Microbiology*, *83*(4), 335-342.
Kapperud, G., & Rosef, O. (1983). Avian wildlife reservoir of Campylobacter fetus subsp. jejuni, Yersinia spp., and Salmonella spp. in Norway. *Applied and Environmental Microbiology*, *45*(2), 375. http://aem.asm.org/content/45/2/375.abstract
Knowles, J. E., Frederick, C., & Knowles, M. J. E. (2016). Package 'merTools'.
Kotula, A., & Sharar, A. (1993). Presence of Yersinia enterocolitica serotype O: 5, 27 in slaughter pigs. *Journal of food protection*, *56*(3), 215-218.
Laukkanen, R., Hakkinen, M., Lunden, J., Fredriksson-Ahomaa, M., Johansson, T., & Korkeala, H. (2010). Evaluation of isolation methods for pathogenic Yersinia enterocolitica from pig intestinal content. *Journal of applied microbiology*, *108*(3), 956-964.
Liang, J., Duan, R., Xia, S., Hao, Q., Yang, J., Xiao, Y., Qiu, H., Shi, G., Wang, S., & Gu, W. (2015). Ecology and geographic distribution of Yersinia enterocolitica among livestock and wildlife in China. *Veterinary microbiology*, *178*(1-2), 125-131.
Martínez, P. O. (2010). Prevalence of enteropathogenic Yersinia in pigs from different European countries and contamination in the pork production chain.
McNally, A., Cheasty, T., Fearnley, C., Dalziel, R. W., Paiba, G. A., Manning, G., & Newell, D. G. (2004). Comparison of the biotypes of Yersinia enterocolitica isolated from pigs, cattle and sheep at slaughter and from humans with yersiniosis in Great Britain

during 1999–2000. *Letters in applied microbiology*, *39*(1), 103-108. https://doi.org/10.1111/j.1472-765X.2004.01548.x

Miller, V. L., Farmer, J. J., Hill, W. E., & Falkow, S. (1989). The ail locus is found uniquely in Yersinia enterocolitica serotypes commonly associated with disease. *Infection and immunity*, *57*(1), 121. http://iai.asm.org/content/57/1/121.abstract

Momtaz, H., Davood Rahimian, M., & Safarpoor Dehkordi, F. (2013). Identification and characterization of Yersinia enterocolitica isolated from raw chicken meat based on molecular and biological techniques. *Journal of Applied Poultry Research*, *22*(1), 137-145.

Murphy, E. G. (2018). Zoonotic Pathogens of Peri-domestic Rodents. *Univeristy of Liverpool*.

Naktin, J., & Beavis, K. G. (1999). Yersinia Enterocolitica and Yersinia Pseudotuherculosis. *Clinics in laboratory medicine*, *19*(3), 523-536.

NCBI, R. C. (2016). Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. *44(D1):D7-D19.* doi:10.1093/nar/gkv1290

Nesbakken, T., Eckner, K., Høidal, H. K., & Røtterud, O.-J. (2003). Occurrence of Yersinia enterocolitica and Campylobacter spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. *International journal of food microbiology*, *80*(3), 231-240.

Neubauer, H., Hensel, A., Aleksic, S., & Meyer, H. (2000). Identification of Yersinia enterocolitica within the Genus Yersinia. *Systematic and Applied Microbiology*, *23*(1), 58-62. https://doi.org/https://doi.org/10.1016/S0723-2020(00)80046-6

Niskanen, T., Waldenström, J., Fredriksson-Ahomaa, M., Olsen, B., & Korkeala, H. (2003). virF-positive Yersinia pseudotuberculosis and Yersinia enterocolitica found in migratory birds in Sweden. *Appl. Environ. Microbiol.*, *69*(8), 4670-4675.

Platt-Samoraj, A., Kończyk-Kmiecik, K., & Bakuła, T. (2021). Occurrence and genetic correlations of Yersinia spp. isolated from commensal rodents in Northeastern Poland. *Pathogens*, *10*(10), 1247.

Platt-Samoraj, A., Żmudzki, J., Pajdak-Czaus, J., Szczerba-Turek, A., Bancerz-Kisiel, A., Procajło, Z., Łabuć, S., & Szweda, W. (2020). The Prevalence of Yersinia enterocolitica and Yersinia pseudotuberculosis in Small Wild Rodents in Poland. *Vector-Borne and Zoonotic Diseases*, *20*(8), 586-592.

Pocock, M., Searle, J., Betts, W., & White, P. (2001). Patterns of infection by Salmonella and Yersinia spp. in commensal house mouse (Mus musculus domesticus) populations. *Journal of applied microbiology*, *90*(5), 755-760.

Powell, L., Cheney, T., Williamson, S., Guy, E., Smith, R., & Davies, R. (2016). A prevalence study of Salmonella spp., Yersinia spp., Toxoplasma gondii and porcine reproductive and respiratory syndrome virus in UK pigs at slaughter. *Epidemiology & Infection*, *144*(7), 1538-1549.

Schaake, J., Kronshage, M., Uliczka, F., Rohde, M., Knuuti, T., Strauch, E., Fruth, A., Wos-Oxley, M., & Dersch, P. (2013). Human and Animal Isolates of Yersinia enterocolitica Show Significant Serotype-Specific Colonization and Host-Specific Immune Defense Properties. *Infection and immunity*, *81*(11), 4013. https://doi.org/10.1128/IAI.00572-13

Shayegani, M., Morse, D., DeForge, I., Root, T., Parsons, L. M., & Maupin, P. S. (1983). Microbiology of a major foodborne outbreak of gastroenteritis caused by Yersinia enterocolitica serogroup O:8. *Journal of clinical microbiology*, *17*(1), 35. http://jcm.asm.org/content/17/1/35.abstract

Shayegani, M., Stone, W. B., DeForge, I., Root, T., Parsons, L. M., & Maupin, P. (1986). Yersinia enterocolitica and related species isolated from wildlife in New York State.

Applied and Environmental Microbiology, *52*(3), 420. http://aem.asm.org/content/52/3/420.abstract

Siembieda, J., Kock, R., McCracken, T., & Newman, S. (2011). The role of wildlife in transboundary animal diseases. *Animal Health Research Reviews*, *12*(1), 95-111.

Sihvonen, L. M., Hallanvuo, S., Haukka, K., Skurnik, M., & Siitonen, A. (2011). The ail gene is present in some Yersinia enterocolitica biotype 1A strains. *Foodborne pathogens and disease*, *8*(3), 455-457.

Tauxe, R., Wauters, G., Goossens, V., Van Noyen, R., Vandepitte, J., Martin, S., De Mol, P., & Thiers, G. (1987). Yersinia enterocolitica infections and pork: the missing link. *The Lancet*, *329*(8542), 1129-1132.

Tauxe, R. V. (2002). Emerging foodborne pathogens. *International journal of food microbiology*, *78*(1-2), 31-41.

Thoerner, P., Bin Kingombe, C. I., Bögli-Stuber, K., Bissig-Choisat, B., Wassenaar, T. M., Frey, J., & Jemmi, T. (2003). PCR Detection of Virulence Genes in Yersinia enterocolitica and Yersinia pseudotuberculosis and Investigation of Virulence Gene Distribution. *Applied and Environmental Microbiology*, *69*(3), 1810-1816. https://doi.org/10.1128/aem.69.3.1810-1816.2003

Van Damme, I., Habib, I., & De Zutter, L. (2010). Yersinia enterocolitica in slaughter pig tonsils: enumeration and detection by enrichment versus direct plating culture. *Food Microbiology*, *27*(1), 158-161.

Virtanen, S., Salonen, L., Laukkanen-Ninios, R., Fredriksson-Ahomaa, M., & Korkeala, H. (2012). Piglets Are a Source of Pathogenic Yersinia enterocolitica on Fattening-Pig Farms. *Applied and Environmental Microbiology*, *78*(8), 3000. https://doi.org/10.1128/AEM.07805-11

Wannet, W. J., Reessink, M., Brunings, H. A., & Maas, H. M. (2001). Detection of pathogenic Yersinia enterocolitica by a rapid and sensitive duplex PCR assay. *Journal of clinical microbiology*, *39*(12), 4483-4486.

Wauters, G. (1979). Carriage of Yersinia enterocolitica serotype 3 by pigs as a source of human infection. *Contributions to microbiology and immunology*, *5*, 249-252.

Weynants, V., Jadot, V., Denoel, P. A., Tibor, A., & Letesson, J.-J. (1996). Detection of Yersinia enterocolitica serogroup O: 3 by a PCR method. *Journal of clinical microbiology*, *34*(5), 1224-1227.

Young, H. S., Dirzo, R., Helgen, K. M., McCauley, D. J., Billeter, S. A., Kosoy, M. Y., Osikowicz, L. M., Salkeld, D. J., Young, T. P., & Dittmar, K. (2014). Declines in large wildlife increase landscape-level prevalence of rodent-borne disease in Africa. *Proceedings of the National Academy of Sciences*, *111*(19), 7036-7041. https://doi.org/10.1073/pnas.1404958111