Rodents, a potential Wildlife Reservoir of *Yersinia enterocolitica* in domestic swine

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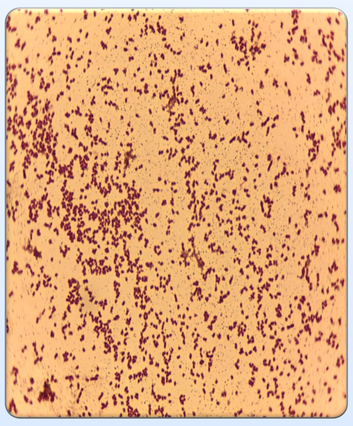

Figure *Y. Enterocolitica* gram stain

Introduction

Yersinia enterocolitica has been identified as one of the four main food-borne public health hazards associated with swine (EFSA, 2011). Pigs are the primary reservoir of human (Powell et all, 2015). However, to develop control measures and lower the incidence of Y. enterocolitica or maintain Yersinia-free herds, better knowledge is needed of possible sources of contamination at the farm level. Wild or peri-domestic rodents may be one such source, since these pests often have free access to pig houses, even under controlled housing schemes rodent control remains difficult.

The aim of this study was to generate a pilot set of data on the prevalence of pathogenic *Y. enterocolitica* in the faeces of wild and peri-domestic rodents collected from pig farms and other peri-domestic locations (Murphy 2019). Then identify pathogenic genes- Ail, Yst and VirF using multiplex PCR as well as 16S Sanger sequencing and mass spectrometry for confirmatory diagnosis, and to further characterise these cultures by conducting serotyping and biotyping of all positive samples.

Figure Trichalose and Xylose positive and negative reactions.

Materials and Methods

- In total, 345 faecal samples from 7 peri-domestic rodent species and 1 species of insectivore were placed into ICT buffer and incubated at 30°C for 36 hours.
- Broth was plated onto CIN agar by the direct plating method and incubated at 30°C for a further 24 hours.
- Colonies were enumerated and suspected *Y. enterocolitica* colonies underwent urease testing and identification via API 20 E.
- Multiplex PCR was carried out to identify pathogenic genes - *ail*, *yst* and *virF,* as well as serotype 03 and 09.
- Additional serotyping for 05, 08 and 27 was undertaken via agglutination.
- Confirmatory identification was carried out via matrix-assisted laser desorption/ionization (MALDI) and 16s Sanger sequencing.
- Confirmed positive samples were biotyped using Bile esculin, Trichalose, Xylose and Indole sugars (ISO,2003).

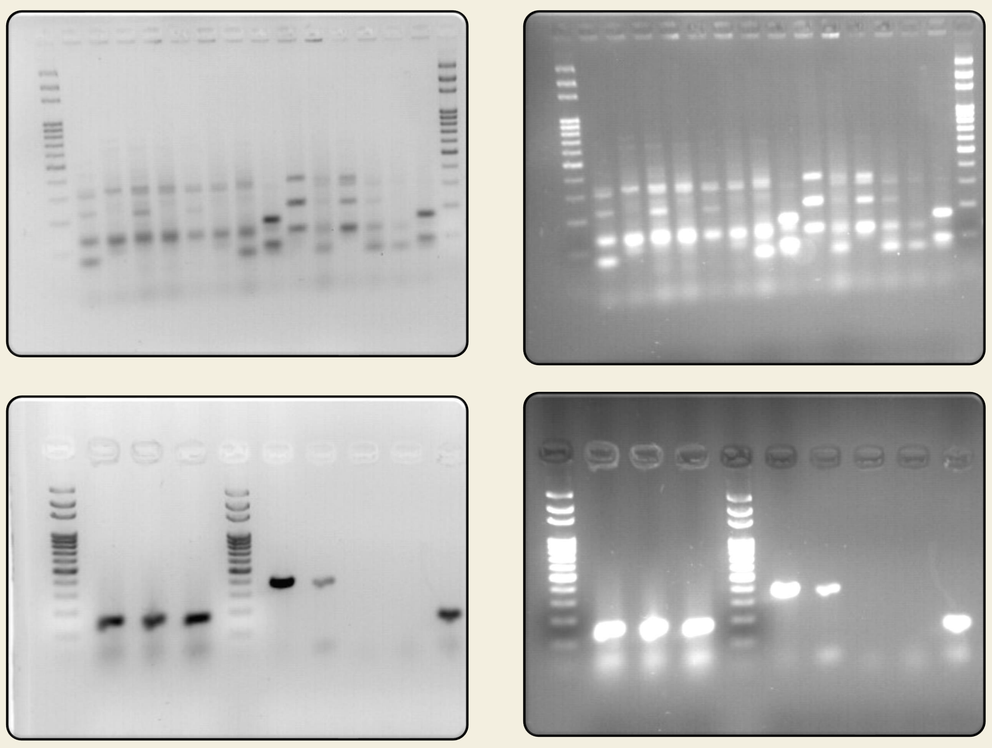

Figure. Multiplex PCR of *Yersinia enterocolitica* showing genes ail (356 KBP), yst (134 KBP) and vir F (231 KBP) (top), 03 (405 KBP) and 09 (181 KBP) Serotypes (bottom).

Results

- All positive samples were 16s sanger sequenced and confirmed as *Y. enterocolitica*.
- The total overall prevalence was 5.50%, individual species prevalence were:
  - Brown rats (*Rattus norvegicus*)*:* 5.70% (n=70)
  - House mice (*Mus musculus*): 0% (n=86)
  - Wood mice (*Apodemus sylvaticus*): 2.70% (n=75)
  - Bank vole (*Myodes glareolus*): 2.1% (n=47)
  - Field vole (*Microtus agrestis*): 29.40% (n=17)
  - Grey squirrel (*Sciurus carolinensis*): 2.80% (n=36)
  - Red squirrel (*Sciurus vulgaris*): 0% (n=9)
  - Common shrew (*Sorex araneus*): 100% (n=1)

No statistically significant pattern of prevalence on farms or locations was seen, with the exception of the wild caught Field Voles from a national park in North Wales where the prevalence was 50% of the sampled population.

Figure Bileesculin positive and negative reactions.

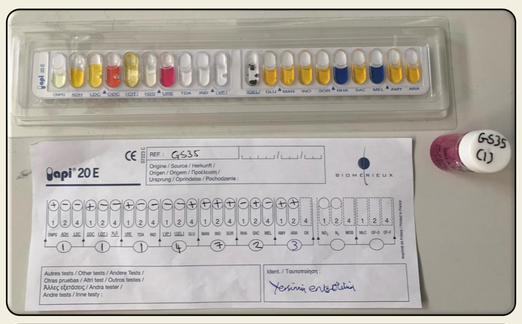

Figure *Yersinia enterocolitica* positive API 20 E and positive urea.

Sample	PCR Genes	Bioserotype
R1	ail, yst	1A/ NI
R10	ail, yst, vir F	1A/NI
R13	vir F, yst	1A/09
R34	yst	1A/09
WM24	NI	1A/09
WM27	vir F, yst	1A/NI
BV16	yst	1A/27
FV1	NI	1A/27
FV4	yst	1A27
FV7	vir F, yst	1A/27
FV9	yst	1A/27
FV10	vir F, yst	1A/27
GS35	NI	1A/NI
SHW1	yst	1A/NI

Table positive *Y. enterocolitica* samples showing their PCR genes and bioserotype.
NI: None identified.

Conclusions

➢Overall prevalence of *Y. enterocolitica* recorded in this study was 5.50%

➢ *Y. enterocolitica* has previously been isolated from brown rats and house mice, however this is the first detection of YE in voles, squirrels and shrews.

➢*Y. enterocolitica* serotypes were isolated in rats, wood mice and field voles. However, all biovars isolated in this study were 1A non-pathogenic.

➢It is therefore unlikely that wild and peri-domestic rodents are a source of infection for pathogenic *Y. enterocolitica* on pig farms.

➢1A was found to have in most isolates ail and yst genes. This was not traditionally the case, however new findings are different

➢However, the source and transmission of Y. enterocolitica infection in pigs is still unclear.

➢Therefore, more work at farm level needs to be done to investigate the epidemiology of this pathogen further.

References

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- Powell, L., Cheney, T., Williamson, E., Smith, R., and Davies, R. (2015). A prevalence study of Salmonella spp., Yersinia spp., Toxoplasma gondii and porcine reproductive and respiratory syndrome virus in UK pigs at slaughter. Epidemiology and Infection, 144(07), 1538-1549.
- ISO (International Organisation for Standards) (2003), Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*. British Standard.