



Isolation of pathogens and drugs resistant genes from crows and their environment

Sheila F. Cubero-Rodríguez¹, David No², Karen Michael², Jack DeLap³, John M. Marzluff³ & Marilyn C. Roberts²

¹URGREAT-MBRS-RISE, School of Sciences and Technology, Universidad del Este, Carolina, PR

²Department of Environmental & Occupational Health Sciences, University of Washington, Seattle, USA

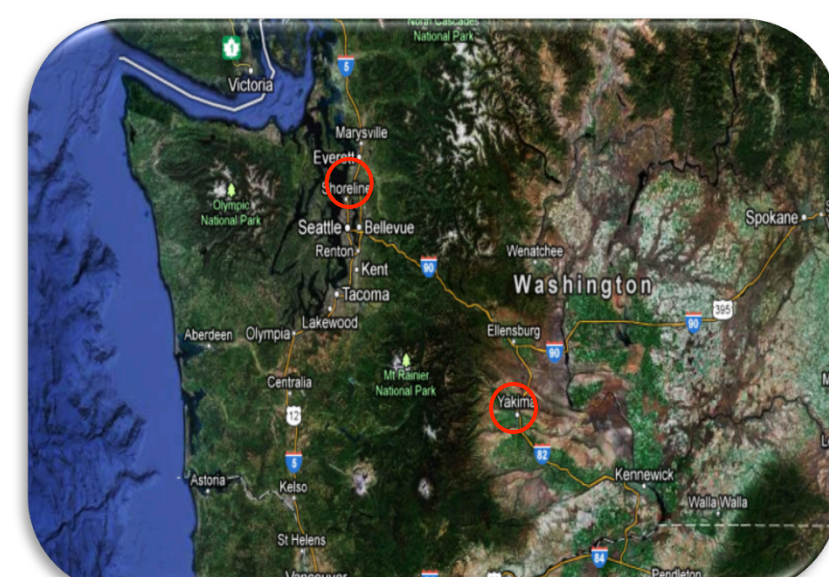
³School of Environmental and Forest Sciences, University of Washington, Seattle, USA



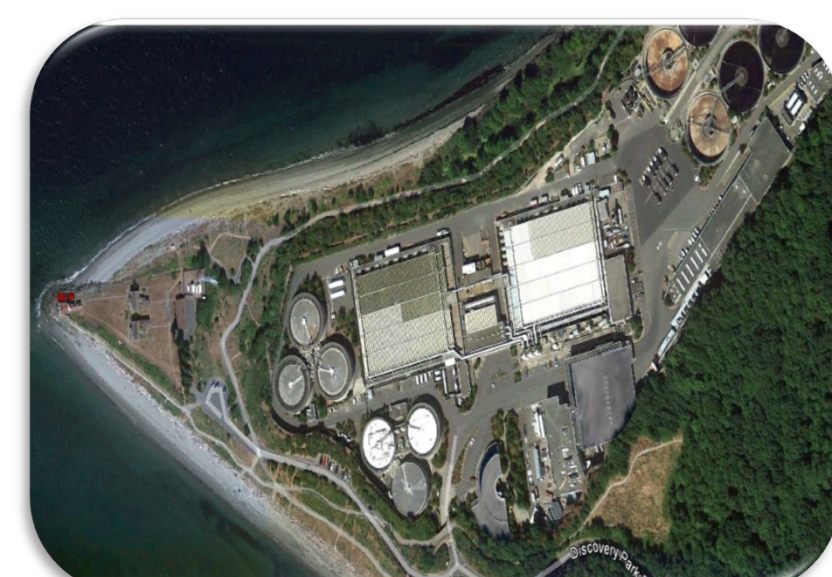
INTRODUCTION

The objective of this study was to determine if we could isolate pathogens and detect antibiotic resistant bacteria and genes in crow feces and their environments (urban wastewater and agricultural cow feces). Crows were captured at wastewater and agricultural locations, and feces were obtained under UW IACUC approval. Primary and secondary wastewater samples were collected, and cow feces obtained from agricultural lands. Twenty-six samples were processed (crows n=20 [14 urban; 6 agricultural], dairy barns n=2 and wastewater n=4) using classical culture methods for *Campylobacter* spp., *E. coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* [MRSA] and vancomycin-resistant *Enterococcus* (VRE) and drug resistant bacteria. By culturing, in the urban setting *Campylobacter* spp. was identified in 21% crows vs. 25% wastewater while at agricultural sites 33% crows vs. 50% cows; *Salmonella* spp. in 14% vs. 75%; *S. aureus* urban 7% vs. 0% and agricultural 50% vs. 50%; and VRE in urban 14% vs. 100% and agricultural 33% vs. 0%. Culture of antibiotic resistant Gram-negative bacteria ranged from 29-57% in the urban crows and 50-100% in wastewater and 17-100% in agricultural crows and 50-100% of cows. Percentage of PCR positive samples for antibiotic resistant genes in urban crows vs. wastewater were identical for macrolide resistant genes *mef*(A) and *erm*(B) [33%], and tetracycline resistant genes *tet*(B) [67%], and *tet*(Q) [33%] while in agricultural areas macrolide resistant genes between crows at 17% and 50%-100% in cows and tetracycline resistant genes *tet*(B) 50% vs. 100%, and *tet*(Q) 0% vs. 100%. The pilot study identified four pathogens and a variety of drug resistant genes in both crow populations. There seems to be differences in what the crows carried in their feces depending on where they lived (urban vs. agricultural) but the total number of samples are too low to determine if this is a real trend.

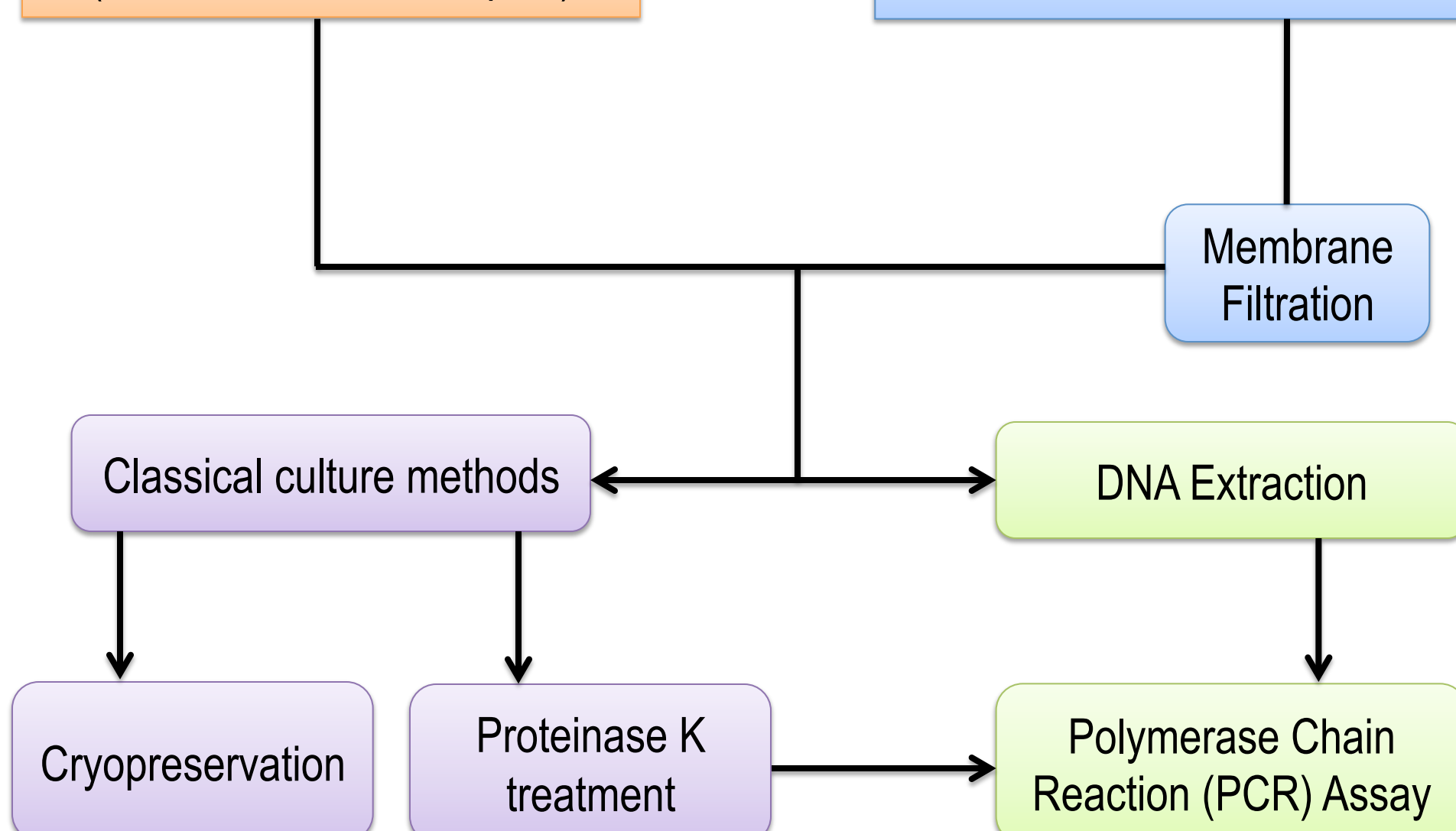
METHODS



Yakima and Monroe dairy farms (crows and cows samples)



West Point Treatment Facility (wastewater samples)



RESULTS

Table 1. Positives urban and agricultural samples in selective media and antibiotic resistant genes present by PCR

| Isolated Bacteria | Urban Environmental Sewage Treatment Samples | | Agricultural Environmental Monroe and Yakima Dairy Samples | |
|---|--|----------------|--|-----------------------|
| | Crows n=14 | Wastewater n=4 | Crows n=6 | Dairy Manure Pile n=2 |
| <i>Campylobacter</i> spp. | 3 | 1 | 2 | 1 |
| <i>Salmonella</i> spp. | 2 | 3 | 0 | 0 |
| <i>Staphylococcus aureus</i> | 1 | 0 | 3 | 1 |
| Ampicillin-resistant <i>E. coli</i> (Am ^r) | 7 | 2 | 2 | 1 |
| Chloramphenicol-resistant <i>E. coli</i> (Cm ^r) | 4 | 3 | 1 | 2 |
| Tetracycline-resistant <i>E. coli</i> (Tc ^r) | 5 | 3 | 3 | 2 |
| Vancomycin-resistant <i>Enterococcus</i> | 2 | 4 | 2 | 0 |
| Gene | Urban Environmental Sewage Treatment Samples | | Agricultural Environmental Monroe and Yakima Dairy Samples | |
| | Crows n=9 | Wastewater n=3 | Crows n=6 | Dairy Manure Pile n=2 |
| <i>erm</i> (B) | 3 | 1 | 1 | 1 |
| <i>mef</i> (A) | 3 | 1 | 1 | 2 |
| <i>mec</i> (A) | 2 | 0 | 0 | 0 |
| <i>tet</i> (B) | 6 | 2 | 3 | 2 |
| <i>tet</i> (M), <i>tet</i> (O), <i>tet</i> (S) | 0 | 0 | 1 | 0 |
| <i>tet</i> (Q) | 3 | 1 | 0 (n=5) | 2 |
| <i>inv</i> (A) (for identification of <i>Salmonella</i>) | 0 | 2 | 0 | 0 |

Fig 1. Percent of pathogens and antibiotic resistant bacteria from urban and agricultural environmental samples

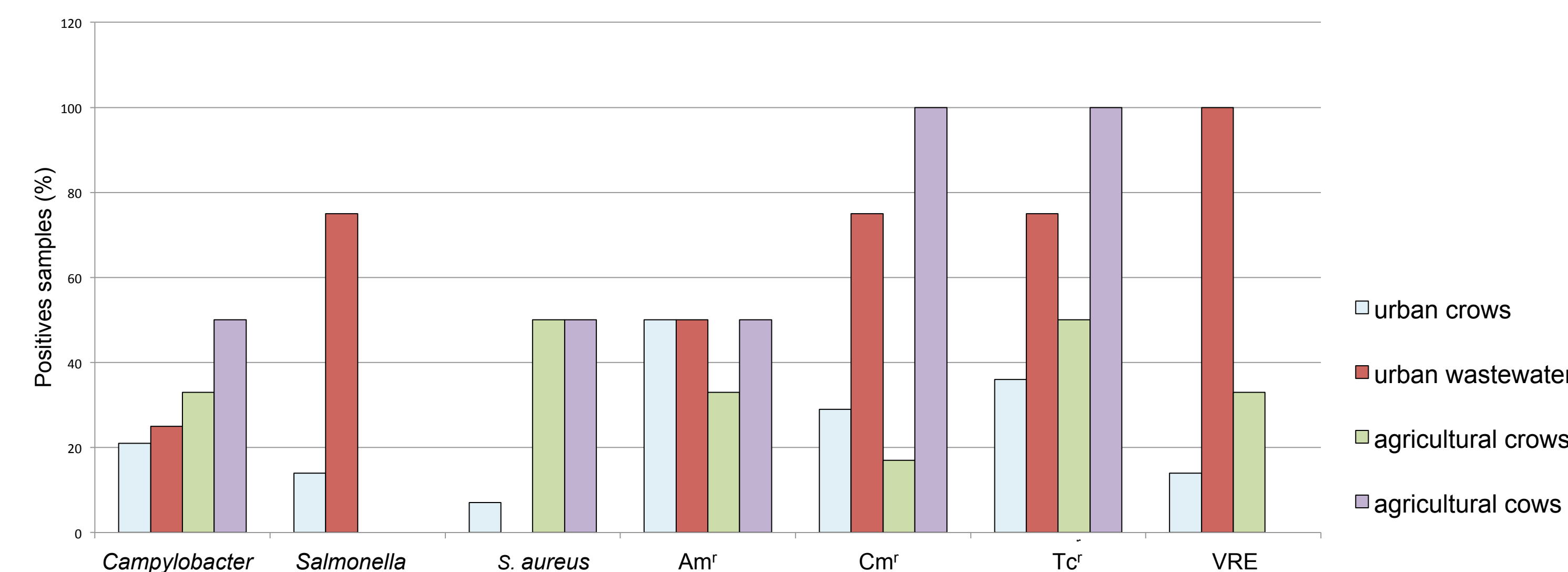
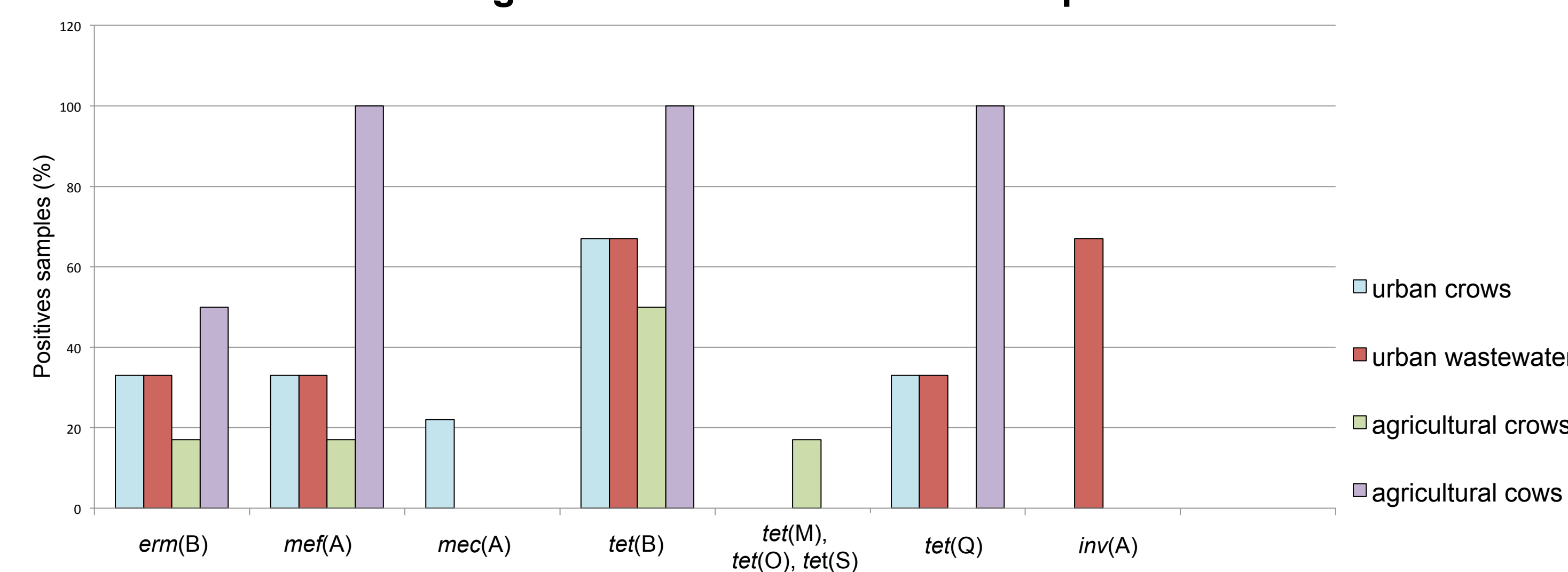


Fig 2. Percent of antibiotic resistant genes present by PCR in urban and agricultural environmental samples



CONCLUSION

- Pathogens (*Campylobacter* spp., *Salmonella* spp., *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus*) were found in crow samples in urban and agricultural setting, which is the first time in crows.
- In both crows population (urban and agricultural) were identified antibiotic resistant bacteria and genes.
- All the samples in both environments presented the following drugs resistant genes: macrolide *mef*(A), *erm*(B) and tetracycline *tet*(B).
- The crow and wastewater samples from urban environmental, show a percentage similarity in *mef*(A), *erm*(B), *tet*(Q) and *tet*(B).
- There seems to be differences in what the crows carried in their feces depending on where they lived (urban vs. agricultural) but the total number of samples are too low to determine if this is a real trend.

FUTURE WORK

- Collect more samples to determine if difference is real between urban and agricultural settings.
- Are pathogens found in crows genetically related to those isolated from their environment?

ACKNOWLEDGMENTS

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