Meat Juice Serology: a Novel Approach to Wild Deer Disease Surveillance

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Abstract

This pilot study aims to pave the way for validation of commercially available ELISA kits for BVD & IBR in wild deer species.

On cattle: Extraction temperature analysis showed no statistically significant mean yield total, *p*=0.22 (*n*=63). BVDV dilution testing showed significant differences *p*<0.001 (*n*=65). IBR S/N values between all tissue showed no statistically significant differences, *p*=0.94 (*n*=105).
On deer: No statistically significant differences were found between all tissue on BVD or IBR ELISA, *p*=0.52 & 0.55 respectively (*n*= 59 & 20).

Introduction

AIM: To enhance surveillance and diagnostic capability of Bovine Viral Diarrhoea Virus (BVDV) & Infectious Bovine Rhinotracheitis (IBR in wild deer

WHY: While Northern Ireland's deer population is rapidly expanding, we have little understanding of their contribution to infectious disease status' in livestock species

HOW: By evaluating Meat Juice Serology (MJS) on commercial ELISA kits for Bovine Diarrhoea Disease Virus (BVDV) & Infectious Bovine Rhinotracheitis (IBR), as an alternative to serum

What is Meat Juice? It's the fluid released from muscle post-mortem. Just think of the fluid gathered in the bottom of shop bought meat packaging!

Why use Meat Juice?

- Collection at abattoir thought to represent a lower biosecurity risk than exsanguination or on-farm visits (Molina et al., 2008).
- Muscle tissue can easily be obtained at the abattoir by personnel without extra training, which makes it relatively easy, cost effective and safe (Loreck et al., 2020).
- Already legislated for in some EU countries, e.g., Salmonella surveillance in Germany (Loreck et al., 2020). Therefore, protocols and logistical considerations already exist.
- Harvesting muscle from culled animals is an easy and effective alternative to drawing blood.
- Previous studies have shown the plausibility of repurposing serum ELISA kits for the cattle muscle fluid matrix (Meemken et al., 2014, Thoms & Probst, 2017).

Methods

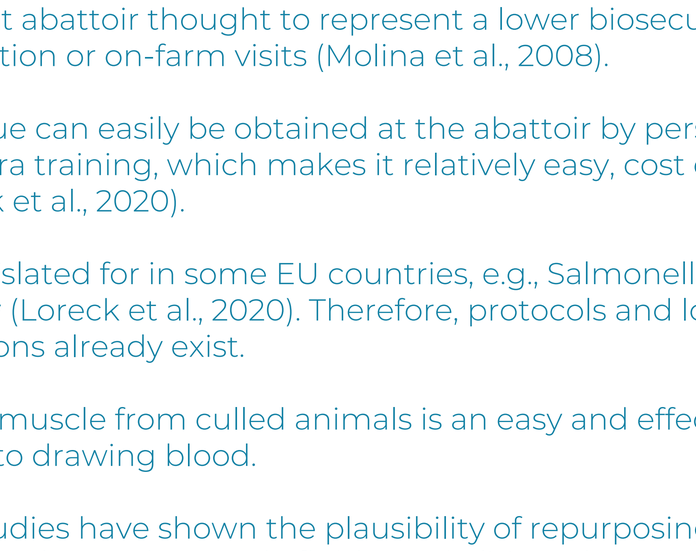

Fig.1 . Illustration of extraction process.: Tissue ~ 3cm3 , frozen in 30ml universal tubes at –80°C or -20°C on arrival. To extract: thawed for 18-24h, upside-down over a polythene funnel inserted into the neck of a 50ml falcon tube. Collected meat juice centrifuged at 4000g for 15m, supernatant stored at -20°C.

- BVDv ELISA: PrioCHECK™ Rum. BVD p80 Kit (ThermoFisher Scientific) to manufacturer's recommended dilution (1:10) and an altered dilution (1:2) on 5 x positive cases and 5 x randomly selected negative cases.
- IBR ELISA: IDEXX IBR/BHV-1 gE Ab ELISA (IDEXX Laboratories Ltd.).

Statistical analysis performed using R Studio (R version 4.0.4 (2021-02-15).

Results

· Extraction Yield Analysis: Total tissue mean at -20°C= 1.81, at - 80°C 1.27. T-test= no statistically significant difference between mean total yields, *p*= 0.22 (95% CI -0.34-1.43)

· BVDv ELISA (Cattle): 1:10 dilution (*n*=103). ANOVA analysis= serum/ meat juice agreement (*p*=0.30). Retests at 1:2 dilution (*n*=31), no significant differences between serum and meat juice samples (*p*=0.34). Paired t-test of dilutions shows significance (*p*<0.001, CI= -32.01 to -9.63).

· IBR ELISA (Cattle): Sample/Negative Ratio (S/N) values between all meat juice/ serum agreeable (*p*= 0.94).

· BVDv ELISA (Deer): No significant between tissue differences (*p*=0.52)

· IBR ELISA (Deer): No significant between tissue differences (*p*=0.55)

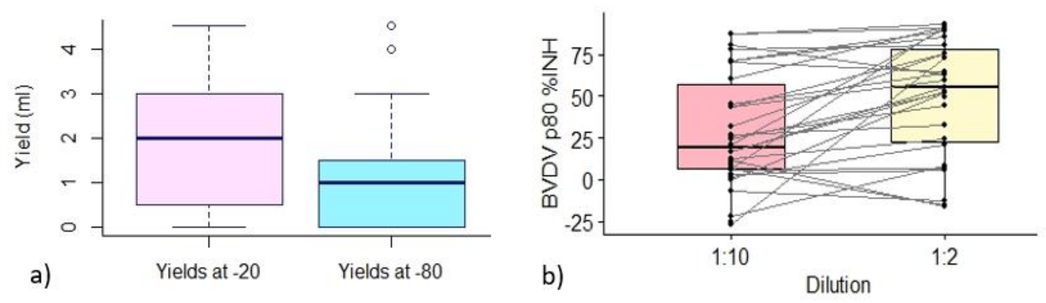

Fig.2 a) Mean meat juice yield from tissue thawed from -20°C and -80°C (*n*=48 & *n*=15 respectively). No statistically significant between temperature difference observed (*p*= 0.22 (95% CI -0.34 -1.43). b) Paired t-test on bovine Meat Juice sample analysed on commercial IDEXX BVDV p80 ELISA at 1:2 and 1:10 dilutions. Overall significance in the difference between %INH values at 1:10 dilution and 1:2 dilution (*p*<0.001, CI= -32.01 to -9.63).

Discussion/Conclusion

- Extraction: Freezing at either temperature appears to be appropriate, hopefully allowing for convenient pre-extraction protocol.
- BVDV: While analyses showed agreement between serum and MJ at both dilution factors, paired sample t-test showed significant differences between dilution factors. Higher dilution may be more appropriate for the Meat Juice matrix.
- IBR: The results indicate that bovine meat juice may be a suitable alternative to serum.
- Deer: Further analysis required on extracted samples (*n*=58, 39 animals) from 2019/20 season, see map (fig.3) for NI deer abundance (orange weighted circles) and cull locations (red). NB: many samples were inappropriate for extraction.
- Positive controls required for validation of test sensitivity and specificity on deer species. gE marker IBR to be replaced with gD for future deer analysis.
- Larger, more homogenous sample populations would add robustness.

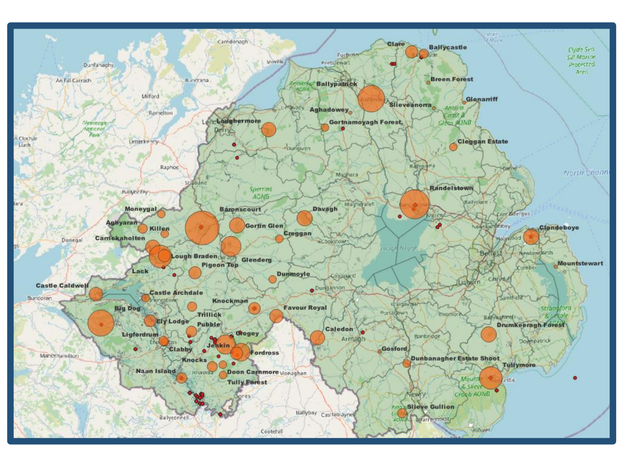

Fig.3. Map of deer abundance locations, data from DAERA Forestry Service FOI request 2020 & deer cull locations 2019/20.

References

Loreck et al., (2020), Use of Meat Juice And Blood Serum With A Miniaturised Protein Microarray Assay, BMC Veterinary Research, vol. 16, 106
Meemken et al., (2014), Establishment Of Serological Herd Profiles For Zoonoses And Production Diseases In Pigs By "Meat Juice Multi-Serology", Preventive Veterinary Medicine, vol. 11(4)3, pp. 589-598
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Thoms B & Probst D (2017), Validation Of The Examination Of Meat Juice Samples For BHV-1, Federal Research Institute for Animal Health, Der Labloeffler News für das Labor, vol 8(2), p. 6.