



PROJECT REVIEW

NAME: Lorna L. Dawson	
INSTITUTE: Newcastle University	FULL TIME Year 3
TITLE: The use of oral fluid from pig populations for the diagnosis and monitoring of infectious disease	
AIMS & OBJECTIVES:	
<p>The overall aim is to develop validated protocols for using porcine oral fluid (OF) for the diagnosis and monitoring of infectious disease. Specific objectives are:</p> <ol style="list-style-type: none"> 1. To further develop laboratory diagnostics for reliable detection of key UK endemic disease agents in oral fluid. 2. To determine factors affecting the composition of porcine oral fluid and how this influences recovery of markers of specific infectious diseases. 3. To develop and test field sampling strategies for pigs housed under the range of UK conditions, including large group, straw based housing systems in which the methodology has not currently been validated. 	

KEY MILESTONES:	TARGET DATE:	ACHIEVED DATE:
<u>Year 1</u>		
Completion of literature review	Feb 2012	March 2012
Training visits completed		
Composition studies on OF initiated	May 2012	May 2012
RT-PCR (for PRRSv) and ELISA (for PRRS & <i>Salmonella</i>) protocols established and validated for OF	Sept 2012	Jan 2013
<u>Year 2</u>		
Protocols for sample handling / storage established	March 2013	August 2013
Representative sampling protocols established for larger pig groups	Sept 2013	Data collected
<u>Year 3</u>		
Field validation of OF diagnostics by parallel sampling with Regional Surveillance serum testing completed.	June 2014	In progress
PhD thesis submitted	Sept 2014	

PROJECT REVIEW AND COMMENTARY:

Methods to preserve viral RNA within swine OF samples have been investigated. Flinders Technology Associates (FTA) cards (GE Healthcare, UK) are treated with a patented formula allowing ambient temperature storage of dried biological samples for subsequent recovery of nucleic acids. An experiment to investigate the ability of FTA cards to store PRRSv-spiked OF samples at ambient temperatures resulted in viral RNA being successfully recovered for up to one month after sample collection, with a detection limit of $1 \times 10^{1.2}$ TCID₅₀/ml. The next step will be to validate this safe, simple method of sample storage/transport using real pen-based OF's collected from pig farms across the UK as part of the OF validation against the current Gold Standard serum.

An experiment aiming to detect anti-Salmonella antibodies in swine OF using a commercial serum ELISA (IDEXX Laboratories, Westbrook ME, USA) was conducted using expected strong and weak positive, as well as expected negative pen-based OF samples on the basis of their corresponding serum antibody results. Various modifications (sample dilution, volume, incubation time/temperature) to the manufacturer's protocol were tested in order to detect the antibodies in swine OF. Overall, anti-Salmonella antibodies could only be detected in pen-based OF samples with a corresponding mean serum S:P of >2.0. Further work involving sample collection from experimentally challenged pigs is required in order to perform Receiver Operated Characteristics analysis and therefore calculate an OF-specific cut off value for positive samples.



Field validation farm visits have commenced, with paired serum and OF samples being collected for detection of anti-PRRS antibodies and viral RNA as part of another BPEX-funded project looking at swine OF diagnostics in UK pigs. All samples will also be screened for anti-Salmonella antibodies once a protocol for OF has been established. Comparison of PRRS antibody detection in 120 pen-OFs with the corresponding serum data have resulted in sensitivity and specificity estimates of 94% and 70% at the pen-level, and 90% and 71% at the whole-farm level, respectively.

A large scale experiment was conducted on a commercial pig farm recruited as part of the aforementioned validation study. The farm is a wean-to-finish straw yard system with pen population sizes of approximately 200 for weaned and 60-100 for finishing pigs. 1, 2, 4 or 8 cotton ropes were presented to each of four weaned and finishing pens of pigs according to a Latin Square design. A sample of approximately 25% of the pigs in each pen were individually marked to allow identification and recording of the total number of marked pigs to engage in chewing activity, as a representation of the total population. Preliminary ANOVA analysis of the data revealed that providing more ropes to pigs resulted in greater OF volumes in both weaned ($F_{(3,12)} = 14.74, P < 0.001$) and finishing ($F_{(3,12)} = 3.81, P = 0.04$) pigs, and that providing more ropes resulted in a greater number of weaned ($F_{(3,6)} = 13.93, P = 0.04$), but not finishing ($F_{(3,6)} = 0.16, P = 0.916$) pigs engaging in chewing activity. On average, the percentage of the marked population to chew on ropes ranged from 40-70% for weaned, and 40-50% for finishing pigs. In both age groups this suggests that OF sampling may give a broader representation of the total population than existing methods involving blood sampling from individual pigs.

POTENTIAL BENEFIT TO INDUSTRY:

The ability to store dried OF samples at ambient temperatures using Whatman FTA cards could provide a safe means for farmers to collect their own biological samples without the need for veterinary assistance, and send these samples to the diagnostic facility without the need for chilling. FTA card dried samples can be sent in the UK postal system as samples are rendered non-infectious upon contact with the card matrix.

The development of an OF assay to detect anti-Salmonella antibodies will provide a quick, easy method for farmers to obtain their herd status without the need for extensive bleeds by veterinarians. This will in turn complement the BPEX ZNCP by introducing a means of farmer-driven Salmonella monitoring during the life of the pigs as opposed to existing monitoring methods such as meat juice testing which occurs after slaughter.

The successful validation of PRRS testing in pen-based OF against blood serum will open a new avenue of disease monitoring possibilities for UK pig producers. Benefits are largely financial, with the reduced veterinary costs due to the non-invasive nature of OF as a diagnostic test medium, as well as the ability to screen larger numbers of animals from one sample, as OF samples are pen-based. It is also possible to collect individual OF samples for instances that may so require such as boar studs. Additionally, OF sampling causes minimal stress to pigs due to its non-invasive nature as opposed to blood sampling which requires snare capture and jugular venepuncture. OF sampling therefore presents substantial welfare, as well as financial advantages to farmers.

A significant number of pig producers do not house their animals in conventional indoor controlled environment systems, but instead adopt straw yards and outdoor systems due to their perceived welfare benefits, and the ability to operate larger groups relatively easily at lower capital cost. The validation of OF sampling methods to reliably collect diagnostically viable and representative OF samples from these large scale, environmentally enriched populations confirms the applicability of the approach across the diversity of current UK production circumstances.

SUPERVISOR: Professor Sandra Edwards and Dr. Georg Lietz

FUNDERS: BPEX

DATED: October 2013

Notes from Seminar: