

An appraisal of the efficacy and feasibility of rapid, on-farm test methods for the detection of campylobacter

Area of research interest: <u>Foodborne pathogens</u> Study duration: 2011-01-01 Planned completion: 1 March 2014 Project code: FS241049A Conducted by: Agri-Food and Biosciences Institute (AFBI)

Background

A rapid, robust, specific, sensitive test for Campylobacter, suited for on-farm use, which enables results to be available in real time, would greatly assist on-farm control by enabling farmers to monitor the outcomes/impact/efficacy of on-farm intervention activities, on-farm management and on-farm biosecurity. Such a test may enable future development of a 'test and schedule' programme, whereby chicken flocks shedding high numbers of Campylobacter would be identified prior to transport and slaughter.

Research Approach

This project assessed the feasibility of using a range of immuno- and nucleic acid-based detection assays to detect Campylobacter in on-farm flock and environmental matrices.

A number of on-farm samples including litter, faeces (composite), dust, air, and dead bird ventral swabs were evaluated to determine which matrix would yield the highest levels of Campylobacter for presentation to detection assay.

It was not anticipated that liquid culture enrichment would take place on-farm in the ultimate test protocol due to health and safety risks to test users (the farmers) and opportunities for cross-contamination.

Physical enrichment methods, including (immuno) magnetic separation, which are commercially available in convenient technology, were assessed for sensitivity, specificity, ease of use and compatibility with detection devices. The outcomes of this early stage work enabled the highest number of Campylobacter to be presented to a suitable detection assay.

Only rapid detection methods at/or close to commercial availability were considered for feasibility of use on-farm. Development of new technologies was not undertaken, however, where available and applied to other pathogens, the technologies were developed for Campylobacter. The primary methods considered in this feasibility study were lateral flow devices, loop-mediated isothermal amplification, an immuno-biosensor and real time PCR assays.

Results

This project investigated the detection of campylobacters on-farm using a range of commerciallyavailable Campylobacter detection assays (lateral flow devices, isothermal DNA amplification assays and real-time PCR assays [RTPCR]).

Initial studies focused on the identification of the optimal broiler house sample type (litter, faeces, boot swab etc.) as determined by culture, followed by an investigation of the optimal boot swab type and demonstration of boot swab stability with time and temperature. To identify negative samples for the study, a broiler house screening methodology utilising boot swab sampling and RTPCR was undertaken. Culture confirmation of the refrigerated samples confirmed Campylobacter contamination of these samples.

Each of the Campylobacter assays were evaluated against a primary panel of Campylobacters (C. jejuni, C. coli and C. lari) and non-Campylobacter type cultures. Assay performance against a panel of Campylobacter culture-positive and -negative broiler house samples were used together with an evaluation matrix, to identify the optimal assay. The assay with the optimal performance was RTPCR. The RT-PCR protocol was further validated by confirmation of its specificity against a more extensive secondary panel of type cultures. The protocol repeatability and reproducibility was determined. In addition the RTPCR assay was evaluated against all cultured field samples (boot swabs) and rolled out for field evaluations by both large and small processors.

Key Findings:

- Tunika boot swabs were the best for collecting campylobacters from litter
- Samples were stable at 4oC for at least 24h before culture
- Samples were stable for at least 4 days at 21oC for RTPCR, which allowed shipping to the lab without refrigeration
- RTPCR was the optimal test method in terms of specificity, sensitivity, reproducibility and cost (approx. £12.00 per sample) including sampling consumables, postage and laboratory test costs
- Although not an on-farm assay, the RTPCR protocol allows results reporting to farmers within 30 h of sample collection in 96% of cases
- The method now has wide scale adoption from both large processor broiler houses (Industry Biosecurity Group) and smaller independent growers (NFU). Around 300 samples are tested each day

Outputs:

The provision to industry of an alternative broiler house Campylobacter test that is not culturebased, and which is rapid, reliable and easy to implement.

Research report

England, Northern Ireland and Wales

PDF

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