Development and field evaluation of a lateral flow test for on-site detection of campylobacter in poultry

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Background

At the time of the project there was no available inexpensive rapid test for campylobacter that could be used on farm.

Lateral flow test strips can be constructed employing a standard sandwich enzyme-linked immunosorbent assay (ELISA). This is not a difficult assay to construct as long as optimal reagents are selected. Performance of the assay simply involves applying a few drops of diluted sample onto a sample application area and then allowing the assay to proceed until a result is seen by the appearance of one or two coloured bands.

A positive result will demonstrate two coloured bands (one in the test zone and one in the control zone) and a negative result will show a coloured band in the control zone only. The intensity of the coloured band in the test zone is an indication of the concentration of *Campylobacter* in the original sample. Therefore, if a reference scoring card can be created it can be used to interpret the intensity of the positive band in an unknown test sample in terms of organisms per ml (or gram).

Research Approach

The objective of this proof-of-concept study was to develop and trial a dipstick-like test strip onto which a few drops of diluted sample could be spotted for quick, easy, inexpensive and on-farm detection of *Campylobacter* spp in chicken.

The approach first developed an immunoassay capable of quickly detecting pathogenic *Campylobacter* spp in chicken faeces in a 96-well microtitre plate using available antibodies and then to port this assay onto a membrane platform to create prototype lateral flow test strips.

A batch of optimised prototype test strips were then manufactured for field testing and for characterisation of basic assay parameters.

It was anticipated that the outputs from this study would be a *Campylobacter* detection test strip that had been field tested and ‘productised’ into a kit form (i.e., containing all the necessary accessories and instructions).

Results

Optimisation assays identified a number of different antibodies which gave a strong signal using a crude direct ELISA and further optimisation identified the most appropriate filter pads, membrane and conjugates. With this basic assay, sample testing using *Campylobacter* spiked poultry faeces was carried out to confirm that the assay could identify *Campylobacter* in the faecal matrix and to identify any problems with the assay design. Testing did confirm that the assay would detect *Campylobacter*in faeces however the level of detection (LOD) of the assay was reduced by 1.5 log, from log10 6.0 to log10 7.5 cfu/mL, when testing was performed using spiked faecal samples in comparison to *C. jejuni* prepared in buffer alone, due to blocking of the filter pad with faecal material.

Using the optimised material, a batch of prototype strips was prepared in two formats, dipsticks and cartridges, for use in lab testing using *Campylobacter*-negative faecal samples spiked with various concentrations of *C. jejuni* and *C. coli.*

The laboratory testing demonstrated that the experimental test strip could detect *C. jejuni* and *C. coli* if faecal samples comprised ?3x107 cfu/g with a CV of 20%.

Further lab testing demonstrated that chicken faeces caused assay interference with a loss of sensitivity of at least 1 log cfu/mL compared to *Campylobacter* in buffer alone. Further work was necessary to optimise the test strip and this was done by replacing the original glass fibre filter with a slightly thicker cellulose based filter and increasing the run time to 15 minutes.

Following optimisation, the assay was rapid (result in <15 minutes), was easy to perform on-site and detected *C. jejuni* and *C. coli* in faeces from chickens with ? 3x107 cfu/g or mL faeces. The test strip required an inoculum of ~107 total cfu in order to produce a strong visible signal.

Following review of the strip performance it was observed that using a portable reader to quantify (and digitalise) the signal reported by the strip, increased strip sensitivity, adding an extra 1 log cfu/mL to its performance. This increased the LOD down to ~106 cfu/mL faeces, which was equivalent to 105 cfu total in the 100µL inoculum that is applied to the test strip.

Field testing demonstrated that although both types of test strips operated correctly, all the test strips reported negative results with faecal samples and with boot swabs in comparison to conventional microbiological methods. This was determined to be due to a lower than expected *Campylobacter* colonisation level in the tested flocks which was lower than the LOD of the test (3x107 cfu/mL (or log10 7.5). This was supported by conventional microbiology data which showed that the average number of *Campylobacter* in the samples tested ranged from log 4.6 – 5.7 cfu/mL (i.e., 4x104 to 5x105 cfu/mL) which are all below 3x107 cfu/mL, the LOD of the test strip.

Further optimisation of the test strip was required to increase the sensitivity by 2.5 – 3 logs in order to detect a signal from low to moderately contaminated samples. Modifications were suggested to increase sensitivity by 3 logs in total including using a biotin-streptavidin signal amplification system and colloidal gold as a label and optimising buffer strength.

A total of 42 experiments were conducted aimed at optimising the sensitivity of the test strip. This resulted in new versions of test strip being developed using latex and colloidal gold nanoparticles as the label, which both proved to increase sensitivity.

The LOD of the latex labelled strip was found to be 1.5 log better than its predecessor thus the LOD improved from log 6.6 to log 5, and experiments with the gold detection conjugate suggested that an LOD of between log 3 and log 4 cfu/mL was possible although this work could not be completed.

The objective of the project was to develop an easy-to-use and low-cost test for rapid on-farm detection of *Campylobacter* in poultry. With the optimised assay a detection level of log 5 cfu/mL was produced with possible further improvement to log 4 cfu/mL. This level of sensitivity would

allow detection of contaminated poultry flocks colonised with low, medium or high levels of *Campylobacter* spp which would meet industry demand.

Research report

England, Northern Ireland and Wales

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