CamCon

Campylobacter control - novel approaches in primary poultry production

Deliverable 3.1.1. Establishment of methods for quantification of airborne *Campylobacter*
Deliverable was due and delivered Month 24 – a revised version was delivered Month 30.

General comments

The inventor of the integrated lab-on-chip (ILOC) technology platform, Ilochip A/S, went bankrupt during the preparation for the CamCon project. We managed to transfer the intellectual property rights to another Danish company, Delta A/S. However, the priorities of Delta A/S shifted towards clinical point-of-care diagnostics and further development of the ILOC technology and air sampling was therefore not made. Technical problems with the equipment, available, experienced during the project resulted in the exclusion of this technology platform from the project. Altogether, this resulted in a re-planning of the work in WP3. Hence, the feasibility of air sampling was validated and the deliverables met as reported below using the Sartorius AirPort MD8 (Sartorius Stedim Biotech GmbH) technology along with conventional boot sock sampling.

A total of four flocks of broilers were followed as planned. Yet, one of the flocks was slaughtered ahead of time due to *Salmonella* infections and another two became *Campylobacter*-positive too late during their life course to allow comprehensive analyses. It means that the data on size distribution in relation to production systems and quantities of airborne *Campylobacter* in relation to size distribution of airborne particle is insufficient for publication. The data from two flocks are reported below and are to be compiled with data achieved from ongoing studies in Poland for publication.

Establishment of methods of quantification of airborne *Campylobacter*

The air sampler used was a Sartorius AirPort MD8 (Sartorius Stedim Biotech GmbH) with a capacity to sample 2 m^3 of air. The air was filtered through disposable gelatin filters provided by the manufacturer. During sampling in the barns, a total of 750 L air was sucked through the gelatin filters in approximately 15 minutes.

DNA from the air samples was prepared in triplicate from 1/8 filter sections by resolving each filter section in 1 mL PBS, pH 8.0, and 25 µL Protex 6L (Genencor). The solution was incubated 1 hour at 45 ^{o}C and then centrifuged for 5 minutes at 3,000 × *g* at ambient temperature. DNA purification was performed using Kingfisher (Thermo LabSystems) according to the procedure recommended by the manufacturer.

A standard serial dilution of *Campylobacter* to produce a standard curve was prepared by use of a fresh 48 hour micro-aerophilic culture of *Campylobacter jejuni* in buffered peptone water (BO0201S, Oxoid). Aliquots of 200 µL were stored at -20^{o}C to provide a stock suspension of uniform quality and concentration. The viable count (colony forming units, CFU) per mL stock suspension of *C. jejuni* was estimated from one of the aliquots prior to storage from a 10-fold dilution series. Once prepared, an aliquot of 200 µL stock suspension was used to prepare a 10-fold serial dilution in PBS and from each dilution, 100 µL was applied to 1/8 section of a gelatin filter. The filter sections representing a serial dilution of the stock suspension were used in each series of qPCR analyses performed on sampled material to provide a quantitative estimate irrespective of inter-test variation.

Quantitative detection of *Campylobacter* was done using the qPCR method of Josefsen et al. (AEM. 2010. 74: 5097-5104)). Standard curves between Ct-values and cell count of *Campylobacter* are shown in 5 repetitions in Fig. 1.

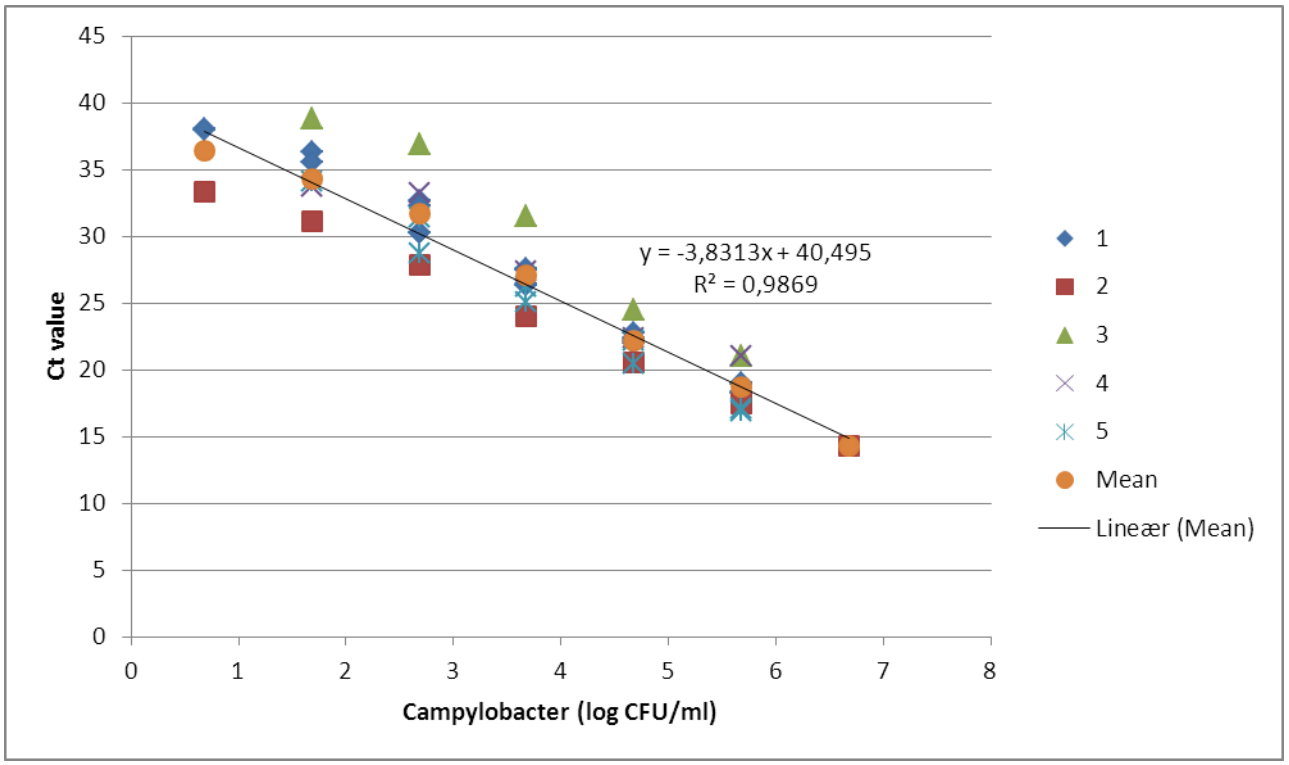

Figure 1. Standard curves produced from five series of DNA extraction from gelatin filters spiked with 100 µL *Campylobacter jejuni* CCUG 11284 (ATCC) from a 10-fold serial dilution of a standard stock suspension. A linear standard curve was drawn based on the mean values of Ct-values at each dilution.

The slope of the standard curve is steeper than normally seen (approximately -3,2) suggesting either that the real dilution factor is less than 10-fold or the DNA purification from the gelatin membranes is not quantitative.

The method of quantification of *Campylobacter* in air is to be published together with quantities of *Campylobacter* in air (D. 3.1.3) observed during these studies and compiled with data from ongoing studies in Poland.