CamCon WP1 Sampling Protocol

8-Farm Longitudinal Study

One standard broiler house selected on each of 8 farms. Every flock in each of these houses to be followed intensively over a 2-year period, each being sampled 1-2 times per week.

1) Bootsock collection.

Collect bootsock samples from study house every day from day 0 (before introduction of chicks), until first thin/slaughter (approx. Day 36) **or** until a positive result is obtained in the lab for one or more bootsocks (we will notify farmers as soon as this happens).
Also collect 1 pair of bootsocks from the ante-room (wear blue plastic bootcovers if you've just dipped your boots to avoid disinfectant on the sample) and 1 from the path leading to the house once weekly (i.e. every time you sample the main house), and 1 from inside every other house on the farm once a week. Place all these in separate plastic bags and return to the lab for processing.

2) Litter collection.

Collect a sample of the litter in the sample house **once a week** by mixing handfuls of litter from around the house together in a plastic bag. Return this to the lab for processing.

Litter will be analysed to test the pH and water content .

All samples from farms or slaughterhouse MUST be given numbers and recorded in the blue lab book in G10.

3) Bootsock processing.

Bootsocks are placed in 200ml sterile saline solution and agitated to loosen the attached matter. Leave the mixture to settle for about 10 minutes. VERY carefully remove **1ml** of the clear upper solution and place into a sterile eppendorf tube, taking care not to take up any faecal material. Centrifuge at 13,000rpm for 7 minutes. Discard the supernatant, leaving a pellet behind. Perform DNA extraction directly from this using a commercial DNA extraction kit (Promega DNA wizard kit), then perform PCR as described below.

4) Faecal sampling.

Once a flock has tested positive through bootsock sampling, we will return the following day to collect individual faecal swabs from the floor of the shed, collecting samples from 30 birds on each occasion. We will continue to collect these swabs every few days, stopping when all 30 are positive. Samples are taken by dipping a sterile cotton swab into the centre of whole faeces which appear fresh (not trampled). Place each swab in a separate sterile ziplock bag and return to the lab.

5) Faeces processing.

Streak each swab directly onto a campylobacter selective agar plate (mCCDA with cefoperazone and amphotericin). Incubate for 48h under microaerobic conditions at 42°C.

After 48h select putative campylobacter colonies (small, round, flat, metallic grey-white colonies, gram-negative, test positive in campy latex agglutination test) and streak each isolate twice onto Columbia agar with 5% defibrinated horse blood. Incubate one plate microaerobically at 42°C for 48h before performing DNA extraction by Chelex 100, then PCR. The second plate should be incubated for 48h in air at 30ºC to test for any air growth (air growth suggests *Arcobacter*).

6) Chelex-100 DNA extraction (from culture only).
Place 300µl chelex-100 solution (20g chelex-100 in 100ml PBS) in an eppendorf for each sample. Collect 1 loopful (approx 5-10µl) of purified colonies from the blood agar plate and mix well with the chelex solution. Heat on a hot block at 95°C for 10 minutes then centrifuge at 13,000 rpm for 2 minutes. VERY carefully remove 50µl of the clear supernatant and mix into 450µl sterile water. Store at 4°C short term or -20°C long term (up to 6 months) for use in PCR.

7) Caeca sampling.
Caeca will be collected from the longitudinal study flocks if the flock has not tested positive for *Campylobacter* by first slaughter age. Collect 10 caeca at first depopulation of the flock, from the production line at the slaughterhouse. In an airflow cabinet, open at the blind end using a hot scalpel (this will sear the outside to avoid false positive results from the outside of the caeca). Collect a 1g of the contents from each using a sterile loop and place each into 9ml MRD. Create serial dilutions of these initial samples (500µl in 4.5ml MRD) and plate each dilution onto mCCDA and incubate in microaerobic conditions for 48h at 42ºC. Perform colony counts on all plates after 48h.

8) Litter processing.
When litter arrives at the lab, immediately test the pH using the pH meter in G10. Once the pH has been recorded, weigh the sample (without the bag) in a white tray labelled with the correct farm name to the nearest 0.01g. Place in a drying oven overnight, then weigh again the next morning. Subtract the dry weight from the fresh weight to give the moisture content in g and calculate as a percentage.

20-farm Longitudinal Study

20 farms selected and every flock from each farm sampled at slaughter.
When a flock from a sample farm is sent to slaughter, a researcher will attend the slaughterhouse and collect 10 caeca from the chickens during processing. These will be tested for *Campylobacter* as in step 7.

Fly Screen Intervention Study

Eight houses on separate farms will be selected for this study. Houses on four farms will be fitted with netting to cover the ventilation and windows in order to prevent the entry of flies into the house. A further four farms will be kept without nets. Bootsock samples will be collected **weekly** from inside the houses on all 8 farms and processed as in step 3. The *Campylobacter* status of these flocks will be monitored.

Up to 50 individual flies (or as many as can be caught in one hour) will also be trapped by hand using a plastic bag. The flies will be speciated by an entomologist and then sampled for *Campylobacter* by placing each fly in 10ml Campylobacter enrichment broth and macerating, then incubating the mixture at 42°C for 24 hours under microaerobic conditions. After 24h 1 loopful of the broth should be streaked onto mCCDA plates and the culture and extraction process continued as in step 5-6.

Fly population study

Collection sacks made from fine mesh netting will be placed over the ventilation inlets of 4 houses and collected weekly. The contents will be sent to Denmark for analysis.

***Campylobacter* detection by PCR**

Using 1µl of the extracted DNA as a template, carry out 25µl multiplex reactions using the following primers:

CjejlpxAF (5' ACA ACT TGG TGA CGA TGT A 3')
lpxAR (5' CAA TCA TGD GCD ATA TGA SAA TAH GCC AT 3')
CcollpxAF (5' AGA CAA ATA AGA GAG AAT CAG 3'), which amplify 331bp (*C. jejuni*) and 391bp (*C. coli*) regions of the *lpxA* gene (Klena *et al*., 2004)

Add 20µl of each primer (at 10mM conc.) to a 2ml tube of ReddyMix (Abgene) to form the mastermix for the reactions.

Use positive controls 012 (C. coli) and 30300 (C. jejuni). For any Arcobacter PCRs, use 30006 (A. Butzleri).

For each reaction, use 22µl mastermix, 1µl BSA and 2µl template DNA.

Run at 94°C for 5 mins, then **30 cycles** of 94°C 1min, 50°C 1min, 72°C 1min. then a final extension of 72°C for 10mins.

Run the samples on a 2% gel for 120 minutes. (Huge gel = 8g agarose in 400ml TAE, med. gel = 5g in 250ml).