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An evaluation of sampling- and culturing methods in the Norwegian action plan against *Campylobacter* in broilers

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Abstract

The Norwegian Action Plan against *Campylobacter* in broilers was implemented in May 2001 with the objective of reducing human exposure to *Campylobacter* through Norwegian broilers. From each flock, samples collected at the farm about one week prior to slaughter, and then again at the slaughter plant, are examined for the presence of *Campylobacter*. All farmers with positive flocks are followed up with bio-security advices. Sampling of broiler products at retail level is also included in the Action Plan. The aim of this study was to evaluate the existing sampling and culturing methods of the Norwegian Action Plan against *Campylobacter* in broilers. The material collected was pooled faecal samples, pooled cloacae samples and caecae samples from individuals. The highest number of positives, from culturing of the pooled faecal samples, the pooled cloacae swabs and the caecae swabs from individuals, were obtained at incubation temperature 41.5 °C. When comparing the results at incubation temperature 37 and 41.5 °C, the faecal samples from the farms demonstrated a high concordance, with a kappa value of 0.88. The results from culturing cloacae swabs and caecae samples from slaughter plant level at two temperatures did not agree very well with a kappa value of 0.21 and moderate value of 0.57, respectively, but were both disconcordant at a level of 0.05. Modelling farm level data indicated that if increasing the number of pooled samples per flock from two (in existing regime) to three, the flock sensitivity increases from 89% to 95%. Modelling of slaughter plant data indicated that three pooled cloacae swabs are needed to identify 90% of the positive flocks. The results from the modelling of caecae data indicated that samples from seven individuals are sufficient to identify 90% of the positive flocks and caecae samples could thus be an alternative to cloacae sampling at slaughter plant level.

Keywords: *Campylobacter*; Action plan; Sampling; Culturing

1. Introduction

The Norwegian Action Plan against *Campylobacter* in broilers; hereafter called the Action Plan was implemented in May 2001 (Anonymous, 2001) with the objective of reducing human exposure to *Campylobacter* through Norwegian broiler meat. The Action Plan was initiated and is coordinated by the Norwegian Zoonosis Centre in close cooperation with the Norwegian Food Safety Authority, the National Veterinary Institute, the Norwegian Institute of Public Health, Norwegian School of Veterinary Science, the Centre for Poultry Science and the poultry industry.

All Norwegian broiler flocks slaughtered before 50 days of age are included in the Action Plan. From each flock 10 fresh faecal droppings are collected (and pooled into two samples of five swabs) from the broiler house some days prior (median = 7 days, range 1–19) to slaughter. These samples are hereafter called farm samples. At the slaughter plants, cloacae swabs from 10 individual chickens per flock are collected (pooled into one sample). These are hereafter called slaughter plant samples. All farmers with positive flocks are followed up with bio-security advisories. Sampling of broiler products at retail level is also included in the Action Plan.

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The results from the first two years of the Action plan (2002–2003) revealed that from the 347 flocks positive at slaughter, 190 (54.8%) had negative farm samples (Anonymous, 2003). This discrepancy between slaughter-plant and farm level sampling could be explained by *Campylobacter* introduction after the farm sample was collected. This is plausible because at that point in time 25% of the bird's lifetime still remains. The sensitivity of the farm level sampling may also be sub-optimal. The birds could also be contaminated during transport from farm to slaughter plant (contaminated crates) or at the plant. Cross contamination of samples could occur at the slaughter plant or in rare cases; in the laboratory.

Almost every flock positive at the farm-level sampling were also positive on the slaughter-level sampling (Anonymous, 2003).

At retail-level sampling, some positive samples originate from flocks identified as negative both at farm-level and at slaughter plant-level samples. This could be due to low sensitivity of the testing at both levels or could be caused by cross-contamination of the chicken products later in the processing.

The bacteriological method used in the Action Plan is a modification of Nordic Committee on Food Analysis method no.119, 2nd ed. 1990 (http://www.nmkl.org/) in regard to the use of an incubation temperature of 37 °C and no pre-incubation. The negative effect of this modification on the sensitivity is partly unknown, but should not be too high in samples with a high number of bacteria (Nachamkin et al., 2000).

The aim of this study was to evaluate the sampling- and culturing methods used in the Norwegian Action Plan against *Campylobacter*, focusing on incubation temperature, sample size and sample type.

2. Materials and method

2.1. Farm-level sampling

A total of 94 flocks within the Action Plan were selected among farms being slaughtered during the high-prevalence period July–August 2003 (Anonymous, 2003), with only one flock included from each farm. In the same two months, a total of 637 flocks were slaughtered in Norway. From each flock 30 extra faecal swabs were collected. Thus from each flock 40 faecal swabs pooled five and five into Cary–Blair medium (Oxoid CM 519) were included in the study. Additionally one recently dead chicken was collected from each flock and transported to the laboratory in order to culture from the caecae for *Campylobacter*. The samples were mailed to the laboratory and the transport time varied between 24–72 h.

2.2. Culturing from farm-level samples

The samples were analysed by the routine method used in the Action Plan against *Campylobacter* (Anonymous, 2003). A sterile swab was put into the material from each pooled sample and subsequently inoculated onto two modified Charcoal Cefoperazone Deoxycholate agar plates (mCCDA, Oxoid). A swab corresponds to about 0.1 g of faecal material. The dead chickens were opened in the abdomen, and the caecae were exposed. Using sterile instruments, one of the caecae was opened and caecae material was plated directly onto two mCCDA. The plates were incubated for 48–72 h in a microaerophilic atmosphere; one plate at 37 °C and one plate at 41.5 °C. *Campylobacter*-like colonies were checked for characteristic motility and morphology by phase contrast microscopy. One positive isolate from each positive flock were sub-cultured on blood agar, whereupon the isolate was identified to species by Catalase reaction, susceptibility for Nalidixin and Cepalothine and hydrolysis of Hippurat, according to standard procedures (On and Holmes, 1991). The possibility of *Arcobacter* was ruled out by sub-culturing aerobically at 30 °C.

2.3. Slaughter plant-level sampling

Nine *Campylobacter* positive flocks were identified through the regular farm-level monitoring in the Action Plan in July 2003. An additional two negative flocks were included in order to investigate whether flocks could have been infected during the time between farm-level sampling and slaughter (one of them originated from a farm with previous positive flocks). From each farm, only one flock was included. Eight of the flocks were slaughtered at one plant while three were slaughtered at another. From each flock cloacae swabs from 40 animals were taken and pooled into four samples in Cary–Blair transport media. Additionally one intact caecum from 10 birds was collected in separate small plastic cups. All the samples, kept cool by ice packs, were mailed to the laboratory. The transport time was 24–72 h.

2.4. Culturing from plant-level samples

The cloacae swabs from the flocks at plant level were cultured by the method described for farm level samples. Presumptive *Campylobacter* colonies were verified according to NMKL 119; Catalase and Oxidase test (Oxidase Reagent, bioMérieux, Marcy/Etoile, France), and hydrolysis of Hippurat (Sodium Hippurat, Sigma). In addition, hydrolysis of Indoxylacetat (Indoxyl Acetate Disk, Remel, Lenaxa, Kansas, USA) and aerobic incubation at 30 °C in order to exclude the presence of *Arcobacter* was carried out.

Buffered Peptone Water (BPV) (10:1 relationship) was added to the plastic cups containing the caecae sample. The cups were then shaken before about 0.1 ml was streaked onto mCCDA with a loop (at room temperature). The culturing continued as described above.

2.5. Software and modelling

The data were stored in Excel (Microsoft, Seattle, WA). Statistical analysis was conducted in Intercooled Stata 8.0 (Stata Corp. College Station, TX). Kappa and McNemar test statistics (Altman, 1997) were calculated for samples cultured at the two different temperatures (collected at farm and