Intestinal colonization of broiler chickens by Campylobacter spp. in an experimental infection study

Consumption of poultry meat is considered as one of the main sources of human campylobacteriosis, and there is clearly a need for new surveillance and control measures based on quantitative data on Campylobacter spp. colonization dynamics in broiler chickens. We conducted four experimental infection trials, using four isolators during each infection trial to evaluate colonization of individual broiler chickens by Campylobacter jejuni over time. Individual and pooled faecal samples were obtained at days 4, 7 and 12 post-inoculation (p.i.) and caecal samples at day 12 p.i. There were large differences between broiler chickens in the number of C. jejuni in caecal and faecal material. Faecal samples of C. jejuni ranged from 4·0 to 9·4 log c.f.u./g and from 4·8 to 9·3 log c.f.u./g in the caeca. Faecal c.f.u./g decreased with time p.i. Most variation in c.f.u. for faecal and caecal samples was attributed to broiler chickens and a minor part to isolators, whereas infection trials did not affect the total variance. The results showed that pooled samples within isolators had lower c.f.u./g compared to the arithmetic mean of the individual samples. There was a significant correlation between faecal c.f.u./g at days 4 and 7 p.i., days 7 and 12 p.i. and for caecal and faecal c.f.u./g at day 12 p.i.

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Contributors: Bahrndorff, S., Garcia Clavero, A. B., Vigre, H., Nauta, M., Heegaard, P. M. H., Madsen, M., Hoorfar, J., Hald, B.
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Towards the production of reliable quantitative microbiological data for risk assessment: Direct quantification of Campylobacter in naturally infected chicken fecal samples using selective culture and real-time PCR

Poultry has been identified as a significant source for human campylobacteriosis which constitutes an important zoonosis and public health problem in many areas of the world. Rapid, direct and accurate quantification of Campylobacter in poultry is essential for the assessment of public health risks and for the evaluation of control strategies implemented in poultry production. The aim of this study was to compare estimates of the numbers of Campylobacter spp. in naturally infected chicken fecal samples obtained using direct quantification by selective culture and by real-time PCR. Absolute quantification of Campylobacter by real-time PCR was performed using standard curves designed for two different DNA extraction methods: Easy-DNA™ Kit from Invitrogen (Easy-DNA) and NucliSENS® MiniMAG® from bioMérieux (MiniMAG). Results indicated that the estimation of the numbers of Campylobacter present in chicken fecal samples was partly dependent on the methodologies used. In general, the numbers of Campylobacter obtained by real-time PCR when extracting DNA using the MiniMAG method were in most cases higher than the numbers of Campylobacter obtained by selective culture and by real-time PCR when using the Easy-DNA method. Although there were differences in terms of estimates of Campylobacter numbers between the methods and samples, the differences between culture and real-time PCR were not statistically significant for most of the samples used in this study.

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Campylobacter vaccination of poultry: Clinical trials, quantitative microbiological methods and decision support tools for the control of Campylobacter in poultry

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Direct Quantification of Campylobacter jejuni in Chicken Fecal Samples Using Real-Time PCR: Evaluation of Six Rapid DNA Extraction Methods

Direct and accurate quantification of Campylobacter in poultry is crucial for the assessment of public health risks and the evaluation of the effectiveness of control measures against Campylobacter in poultry. The aim of this study was to assess several rapid DNA extraction methods for their effectiveness for the direct quantification (without enrichment) of Campylobacter jejuni in chicken fecal samples using real-time PCR. The presence of inhibitory substances in chicken fecal samples may reduce or even completely impede the PCR amplification process making quantification very difficult. Six rapid DNA extraction methods were compared based on their limit of detection, efficiency, reproducibility, and precision. Standard curves were designed for all the methods tested in order to assess their performance on the direct quantification of C. jejuni in chicken fecal samples. As a result of this study, the Easy-DNA (Invitrogen) method generated lower Ct values, the best amplification efficiency (AE = 93.2 %) and good precision (R squared = 0.996). The method NucleoSpin® Tissue was able to detect samples spiked with the lowest Campylobacter concentration level (10 CFU/ml) but the amplification efficiency was not optimal (AE = 139.5 %). DNA extraction methods Easy-DNA Invitrogen, MiniMAG® and NucleoSpin® Tissue produced good real-time PCR reproducibility generating standard deviations from 0.3 to 0.8 between replicates.

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Integration of Epidemiological Evidence in a Decision Support Model for the Control of Campylobacter in Poultry Production

The control of human Campylobacteriosis is a priority in public health agendas all over the world. Poultry is considered a significant risk factor for human infections with Campylobacter and risk assessment models indicate that the successful implementation of Campylobacter control strategies in poultry will translate on a reduction of human Campylobacteriosis cases. Efficient control strategies implemented during primary production will reduce the risk of Campylobacter introduction in chicken houses and/or decrease Campylobacter concentration in infected chickens and their products. Consequently, poultry producers need to make difficult decisions under conditions of uncertainty regarding the implementation of Campylobacter control strategies. This manuscript presents the development of probabilistic graphical models to support decision making in order to control Campylobacter in poultry. The decision support systems are constructed as probabilistic graphical models (PGMs) which integrate knowledge and use Bayesian methods to deal with uncertainty. This paper presents a specific model designed to integrate epidemiological knowledge from the United Kingdom (UK model) in order to assist poultry managers in specific decisions related to vaccination of commercial broilers for the control of Campylobacter. Epidemiological considerations and other crucial aspects including challenges associated with the quantitative part of the models are discussed in this manuscript. The outcome of the PGMs will depend on the qualitative and quantitative data included in the models. Results from the UK model and sensitivity analyses indicated that the financial variables (cost/reward functions) and the effectiveness of the control strategies considered in the UK model were driving the results. In fact, there were no or only small financial gains when using a hypothetical vaccine B (able to decrease Campylobacter numbers from two to six logs in 20% of the chickens with a cost of 0.025 £/chicken) and reward system 1 (based on similar gross profits in relation to Campylobacter levels) under the specific assumptions considered in the UK model. In contrast, significant reductions in expected Campylobacter numbers
and substantial associated expected financial gains were obtained from this model when considering the reward system 2 (based on quite different gross profits in relation to Campylobacter levels) and the use of a hypothetical cost-effective vaccine C (able to reduce the level of Campylobacter from two to six logs in 90% of the chickens with a cost of 0.03 £/chicken). The flexibility of probabilistic graphical models allows for the inclusion of more than one Campylobacter vaccination strategy and more than one reward system and consequently, diverse potential solutions for the control of Campylobacter may be considered. Cost-effective Campylobacter control strategies that can significantly reduce the probability of Campylobacter introduction into a flock and/or the numbers of Campylobacter in already infected chickens, and translate to an attractive cost-reward balance will be preferred by poultry producers.

The development of probabilistic graphical models to assist on strategic decisions for the control of Campylobacter in poultry

Design and data analysis of experimental trials to test vaccine candidates against zoonotic pathogens in animals: the case of a clinical trial against campylobacter in broilers
Estimation of the variation that can be attributed to different levels in a clinical trial of a vaccine

This presentation is focusing on the interaction between the experimental design of a vaccination trial and appropriate data analysis using a trial of a vaccine against Campylobacter in broilers as an example. This study was designed using four rotations with eight isolators per rotation (10 chickens per isolator). Treatment was administered at isolator level on day 14 (vaccine or placebo). The broilers were inoculated with Campylobacter jejuni at day 31 and slaughtered at day 42. The numbers of Campylobacter (cfu/g) were obtained in the laboratory using selective cultivation methods and log transformed to obtain a Gaussian distribution. Initially, the effect of the vaccine was analyzed using all data in a t-test. Subsequently, the t-test was stratified by rotation. Finally, mixed linear models were used, taking into account the physical hierarchical setup of the trial. Results from the t-test indicate an effect of the vaccine, whereas the result obtained from the complex model indicated high variability between birds and isolators but not significant vaccine effect. The apparent observed differences between vaccinated and placebo groups in the t-tests could be attributed to the variation between incubators. Broilers in the same isolator had more equal numbers of C. jejuni compared to chickens in other incubators. It is possible that chickens in the same isolator re-infect each other with Campylobacter. In this study, the design effect was considerable, reducing the effective sample size (67 animals instead of the 290 animals included). The clustered design used in this trial was trying to emulate the clustering effect found in broiler flocks and farms.
Estimation of the variation that can be attributed to different levels in a clinical trial of a vaccine against Campylobacter in broilers

This presentation is focusing on the interaction between the experimental design of a vaccination trial and appropriate data analysis using a trial of a vaccine against Campylobacter in broilers as an example. This study was designed using four rotations with eight isolators per rotation (10 chickens per isolator). Treatment was administered at isolator level on day 14 (vaccine or placebo). The broilers were inoculated with Campylobacter jejuni at day 31 and slaughtered at day 42. The numbers of Campylobacter (cfu/g) were obtained in the laboratory using selective cultivation methods and log transformed to obtain a Gaussian distribution. Initially, the effect of the vaccine was analyzed using all data in a t-test. Subsequently, the t-test was stratified by rotation. Finally, mixed linear models were used, taking into account the physical hierarchical setup of the trial. Results from the t-test indicate an effect of the vaccine, whereas the result obtained from the complex model indicated high variability between birds and isolators but not significant vaccine effect. The apparent observed differences between vaccinated and placebo groups in the t-tests could be attributed to the variation between incubators. Broilers in the same isolator had more equal numbers of C. jejuni compared to chickens in other incubators. It is possible that chickens in the same isolator re-infect each other with Campylobacter. In this study, the design effect was considerable, reducing the effective sample size (67 animals instead of the 290 animals included). The clustered design used in this trial was trying to emulate the clustering effect found in broiler flocks and farms.

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Quantitative microbiological data analysis of a Campylobacter vaccination trial
Campylobacter jejuni is considered the main pathogen causing human campylobacteriosis and poultry has been identified as one of the main risk factors. Strategies that aim to control Campylobacter in poultry such as vaccination strategies could reduce the incidence of human campylobacteriosis. The objective of the present trial was to assess whether or not a vaccine candidate could give a 2 logs reduction of the numbers of Campylobacter in broilers. Sample size calculations indicated the use of 400 animals (200 vaccinated and 200 controls). The experiment was conducted in four different rotations using 8 incubators per rotation with 10 chickens in each incubator. The vaccination treatment was randomly assigned at incubator level. Broilers were challenged with C. jejuni at day 31 and faecal/caecum samples were collected at slaughter at day 42 and processed in the laboratory. To illustrate the importance of analysing the data in accordance with the setup of the study, the data was analysed both without and with taking the nested design into account. Initially, the effect of the vaccine was analyzed using all data in a t-test. Subsequently, the t-test was stratified by rotation. Finally, mixed linear models were used, taking into account the physical hierarchical setup of the trial. Results from the t-test indicate an effect of the vaccine, whereas the result obtained from mixed linear models indicated high variability between birds and isolators but not significant vaccine effect. The apparent observed differences between vaccinated and placebo groups in the t-tests could be attributed to the variation between incubators. Broilers in the same isolator had more equal numbers of C. jejuni compared to chickens in other incubators. It is possible that chickens in the same isolator re-infect each other with Campylobacter.

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**Bibliographical note**
The use of probabilistic graphical models (PGMs) to develop a cost-effective vaccination strategy against Campylobacter in poultry

Human campylobacteriosis represents an important economic and public health problem. Campylobacter originating from feces of infected chickens will contaminate chicken meat posing a risk to the consumer. Vaccination against Campylobacter in broilers is one probable measure to reduce consumers’ exposure to Campylobacter. In this presentation we focus on the development of a computerized decision support system to aid management decisions on Campylobacter vaccination of commercial broilers. Broilers should be vaccinated against Campylobacter in the first 2 weeks of age. Therefore, the decision about vaccination needs to be made usually before Campylobacter is introduced in the flock. In fact, there is uncertainty regarding the introduction of Campylobacter into the flock that needs to be taken into account in the decision making process. Probabilistic Graphical Models (PGMs) integrate knowledge from diverse sources and can be used as decision support systems under conditions of uncertainty. The relationships between different entities in the model can be designed and conditional probability distributions are used to define the strength of these relationships. Important microbiological, epidemiological and economic factors (cost-reward functions) have been included in the models. The final outcome of the models is presented in probabilities of expected level of Campylobacter and financial terms influenced by the decision on vaccination. For example, if the best decision seems to be to vaccinate, the outcome will be expressed as the most probable number of Campylobacter in broilers and a cost-profit balance. There are other factors that could be considered increasing the complexity of the models, but we need to balance model efficiency with simplicity and usefulness for poultry managers to run the model as a tool for decision making on vaccination.

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Projects:

Development of a decision tool for vaccination against Campylobacter in poultry
Garcia Clavero, A. B., PhD Student, National Food Institute
Vigre, H., Main Supervisor
Christensen, L. S., Supervisor
Læsø Madsen, A., Supervisor
Pedersen, K., Examiner
Rådström, G. P., Examiner
Pielaat, A., Examiner
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CamVac: CamVac: Campylobacter vaccination of poultry
The main objective of this project is to develop a cost-effective vaccination strategy for the poultry production, hereby reducing the colonization of Campylobacter in both parental and broiler flocks.

Vaccination is one of the few measures that can be applied to reduce the colonization of Campylobacter in free range organic poultry. The project aims to identify a vaccination strategy based on reduction, since risk assessment studies have shown that a 2 log reduction of colonization in poultry can reduce the risk of human infection by 30 times.

Hoorfar, J., Project Coordinator, National Food Institute, Division of Food Microbiology
Madsen, M., Project Manager, Dianova A/S
Bang-Berthelsen, I., Project Participant, National Food Institute, Division of Food Microbiology
Christensen, L. S., Project Participant, National Food Institute
Josefsen, M. H., Project Participant, National Food Institute, Division of Food Microbiology
Vigre, H., Project Participant, National Food Institute, Division of Epidemiology and Microbial Genomics
Garcia Clavero, A. B., Project Participant, National Food Institute, Division of Epidemiology and Microbial Genomics
Saunders, I., Project Participant, TD Vaccines
Darsley, M., Project Participant, TD Vaccines
Lund, M., Project Participant, University of Copenhagen
Sandøe, P., Project Participant, University of Copenhagen
Wagenaar, J., Project Manager, Utrecht University
Joens, L., Project Manager, University of Arizona
Cooper, K., Project Participant, University of Arizona
Madsen, A., Project Participant, Hugin Expert A/S
Hald, B., Project Participant, National Food Institute, Division of Food Microbiology
Bahmdorff, S., Project Participant
Heegaard, P. M. H., Project Participant, National Veterinary Institute
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