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Analysis of farm specific risk factors for *Campylobacter* colonization of broilers in six European countries

H.M. Sommer[a,b,*], B. Borck Høg[b], L.S. Larsen[b,1], A.I.V. Sørensen[b], N. Williams[c], J.Y. Merga[c], M. Cerdà-Cuéllar[d], S. Urdaneta[d], R. Dolz[d], K. Wieczorek[e], J. Osek[e], B. David[f], M. Hofshagen[f], M. Jonsson[f], J.A. Wagenaar[g], N. Bolder[g], H. Rosenquist[b]

[a] *Division of Data Analysis and Statistics, DTU Compute, Technical University of Denmark, Building 324, DK-2800 Kgs. Lyngby, Denmark*
[b] *Division of Risk Assessment and Nutrition, The National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark*
[c] *Zoonotic Infections of People, Pigs and Poultry Group, Department of Epidemiology and Population Health, Institute of Infection and Global Health, Leahurst Campus, University of Liverpool, Neston CH64 7TE, UK*
[d] *Centre de Recerca en Sanitat Animal (CReSA), IRTA, Campus de la Universitat Autònoma de Barcelona, 08193-Bellaterra (Cerdanyola del Vallès), Barcelona, Spain*
[e] *Department of Hygiene of Food of Animal Origin, National Veterinary Research Instiute, Partyzantow 57, 24-100 Pulawy, Poland*
[f] *Norwegian Veterinary Institute, Pb 750 Sentrum, N-0106 Oslo, Norway*
[g] *Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.165, 3508 TD Utrecht, The Netherlands*

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ABSTRACT

This study presents on-farm risk factors for the colonization of broiler flocks with *Campylobacter* based on comparable data from six European countries: Denmark, the Netherlands, Norway, Poland, Spain, and the UK. The study includes explanatory variables from a large questionnaire concerning production, farm management procedures and farm conditions, climate data on mean temperature, sunshine hours, and precipitation, as well as data on *Campylobacter* status of broiler flocks. All together the study comprises data from more than 6000 flocks. The data were analysed using a generalized linear model. Due to a large number of parameters, some collinearity and relatively many missing values, the model was analysed by a method using all available cases at each step in the modelling process. The modelling process includes backwards elimination and forward selection. Several approaches were furthermore explored by applying different strategies for categorizing explanatory variables and for selecting and eliminating variables in the model.

Despite national differences in broiler production, common risk factors for *Campylobacter* colonization of broiler flocks were identified across all six countries. These were generally related to inadequate biosecurity. Identified risk factors were: broiler houses older than 15 years, absence of anterooms and barriers in each house, shared tools between houses, long downtime, and drinker systems with bells or cups. Also, the risk of broiler flocks becoming colonized with *Campylobacter* was clearly affected by country. In descending order, broiler flocks were more likely to be colonized in Poland, the UK, Spain, the Netherlands, Denmark and Norway due to country specific factors that could not be explained by the identified risk factors or any other variables from the questionnaire. The seasonality observed for prevalence values was described by the monthly mean temperature reported in the study, i.e. the higher the temperature, the higher the prevalence of positive flocks.

1. Introduction

Many attempts have been made to understand the epidemiology of *Campylobacter* in broilers in search of effective control measures for preventing *Campylobacter* colonization of indoor commercial broiler flocks. *Campylobacter* can be transferred to humans e.g. via broiler meat or via the environment being contaminated by feces from production animals such as for example broilers and cattle and thereby cause campylobacteriosis, which is the most common cause of foodborne gastrointestinal illness in the EU and the rest of the industrialized world (EFSA and ECDC, 2015; WHO, 2012). Studies have shown that the main route of introduction of *Campylobacter* into broiler flocks is from

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[*] Corresponding author.
E-mail address: helle.m.sommer13@gmail.com (H.M. Sommer).

[1] Present address: Statens Serum Institut, 5 Artillerivej, 2300 Copenhagen S, Denmark.

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the environment outside the broiler house due to one or more breaches in biosecurity (Adkin et al., 2006; Hald et al., 2000; Lyngstad et al., 2008). Hence, controlling *Campylobacter* in housed birds is primarily a question of strict management practices with a high level of biosecurity and broiler houses that are closed off to the environment. There are, however, many factors involved in achieving a high level of biosecurity and some are more important than others. The most important risk factors are those, where the risk of carrying *Campylobacter* into the broiler houses is greatest, for example by contaminated air, shoes, clothes, flies, etc.

Previous work from Northern Europe has shown that housed flocks are significantly more likely to be *Campylobacter*-positive during the summer period (Jonsson et al., 2012; Jore et al., 2010), when ambient temperatures are high compared with the winter period. This is partly related to increased airborne transmission of *Campylobacter* due to high airflows into the houses during warm periods (Hald et al., 2008). The bacteria may be present in dust or, more importantly, in insects (Bahrndorff et al., 2013; Hald et al., 2007). Climatic factors may, therefore, partly explain the higher *Campylobacter* prevalence in flocks in parts of Eastern and Southern EU (EFSA, 2010), where ambient temperatures are higher and where there is a constant requirement for higher airflow through the houses. Given variations in climate and broiler flock prevalence across Europe, we speculated if risk factors for flock colonization were similar or different in Northern, Eastern and Southern Europe. To our knowledge, this has not previously been investigated in one study.

Comprehension and awareness of country specific risk factors can guide the choice of the preventive measures in a country to ensure optimal effect, and thus lead to safer broiler meat production within the country. Identification of common risk factors that are applicable in all EU countries is also vital, given the EU-wide nature of broiler production and marketing and the high incidence of campylobacteriosis across the EU.

The objective of this study was therefore to study on-farm risk factors for the colonization of broiler flocks with *Campylobacter* based on comparable data from six European countries. Previously, we analyzed risk factors in two Northern European countries (Denmark and Norway) (Høg et al., 2016). In the present study, we expand the risk factor study to also include data from the Netherlands, Poland, Spain and the UK, and we included climate related variables to the list of risk factors. The study was designed to investigate risk factors related to climate, geography and on-farm management practices in housed broiler flocks (the most common type of broiler production in EU (EFSA, 2011). The analysis was based on a method that uses all available cases at each step in the modelling process (backwards elimination and forward selection) (Sommer et al., 2013). For every fifth step of backwards elimination a forward selection was run to include earlier eliminated variables if p-values were less than 0.10.

2. Materials and methods

2.1. Questionnaire, Campylobacter status and climate data

The farm specific variables were obtained through a standardized questionnaire including 43 questions concerning production, farm management procedures, farm conditions, etc. Further information on the questionnaire can be found in Høg et al. (2011), where the questionnaire is also presented in full. Some questions were excluded due to difficulties in interpretations or too little variation in the responses.

In Denmark and Norway, *Campylobacter* data for flocks on farms that responded to the questionnaire were obtained through national surveillance programmes (Høg et al., 2016). In Norway, only flocks slaughtered from May to October in 2010 and 2011 were tested for *Campylobacter,* in total 1400 flocks. In Denmark, full annual datasets were obtained from 2010 and 2011, in total 3864 flocks. *Campylobacter* flock data from Poland, Spain and the UK were obtained through a two-year longitudinal study within the period 2011–2013, where all flocks in the study houses were tested for *Campylobacter*. Furthermore, *Campylobacter* results were obtained from 276 flocks from Poland, 201 flocks from Spain and 219 flocks from the UK. In the Netherlands, *Campylobacter* status was collected from 221 flocks during 2012 and 2013. All flocks were tested prior to or at the time of catching the first batch of birds from the flock (first thin). All flocks from Denmark, Norway and the Netherlands were sampled on farm by boot swabs and tested using PCR, as described by Lund et al., (2004). In Poland, Spain and the UK, caeca from 10 birds per flock were sampled at the slaughterhouse and pooled before microbial analysis; isolation and confirmation of *Campylobacter* organisms in caecal contents were undertaken as described in ISO 10272-1:2006. At least one *Campylobacter* isolate per batch was speciated using phenotypic methods as described in ISO 10272-1:2006 or by PCR as described by Klena et al., (2004).

Climate data were collected from weather stations as close as possible to the farms included in the study. This meant that two-three climate datasets were obtained from Norway, Poland, Spain and the UK. However, due to small country size for Denmark and the Netherlands, only one data set per country was included for these two countries. Climate data were matched to each flock by the month of slaughter. Three climate variables were chosen to represent the climate; monthly outside mean temperature, monthly total precipitation, and monthly total sunshine hours. These three variables were chosen, because a preliminary analysis of the Danish data had shown their ability to describe the seasonality in the broiler flocks prevalence (data not shown).

2.2. Data preparation

Questionnaire data were prepared for analysis to improve data quality and to maximize the number of data and variables in the final model. This step was essential for running the model with as many variables as possible. Wherever reasonable, missing values were filled in, and the number of parameters was reduced by merging variables or categories. Furthermore, highly correlated variables were excluded.

2.2.1. Merging

To reduce the number of parameters, some of the categories within variables were merged, especially for variables with a large number of categories and many combinations of these. Variables were merged based on expert's opinions and the empirical prevalence estimates. If experts suggested categories to be merged, but the empirical prevalence estimates were largely different, then the categories were not merged. Two questions had a hierarchical structure and were merged in order to avoid collinearity, e.g. the merging of variables concerning downtime between flocks (*Using downtime (yes/no)* and *Duration of downtime*). If no downtime, the duration of downtime was set to zero and, thus, became part of the variable *Duration of downtime* (from now on referred to as *Downtime*).

2.2.2. Collinearity

Correlation between two or more variables (multicollinearity) may cause problems running the model as a consequence of an unsuccessful approximation to the inversed Hessian matrix (Altman et al., 2004). In this study, we removed one of the highly correlated sets of variables from the model initially (to make the model run) and later allowed the removed variables to re-enter the model in the forward selection.