International Journal of Infectious Diseases 28 (2014) 74–79

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Campylobacter, Salmonella, and Yersinia antibodies and pregnancy outcome in Danish women with occupational exposure to animals

Bjørn Kantsø[a,*], Anne-Marie Nybo Andersen[b], Kåre Mølbak[c], Karen Angeliki Krogfelt[d], Tine Brink Henriksen[e], Stine Yde Nielsen[f,g]

[a] Microbiological Diagnostics and Virology, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen, Denmark
[b] Section of Social Medicine, Department of Public Health, University of Copenhagen, Copenhagen, Denmark
[c] Infectious Disease Epidemiology, Statens Serum Institut, Copenhagen, Denmark
[d] Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark
[e] Perinatal Epidemiology Research Unit and Department of Paediatrics, Aarhus University Hospital, Skejby, Denmark
[f] Department of Occupational Medicine, Regional Hospital West Jutland, Herning, Denmark
[g] Department of Clinical Microbiology, Aarhus University Hospital, Skejby, Denmark

ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 18 June 2014
Accepted 27 June 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Campylobacter
Salmonella
Yersinia
Miscarriage
Preterm birth
Small for gestational age (SGA)

SUMMARY

Background: The aim of this study was to determine antibody titres against Campylobacter, Salmonella, and Yersinia in a population-based cohort of pregnant women in Denmark in order to evaluate adverse pregnancy outcomes (miscarriage, preterm birth, and small for gestational age) in relation to occupational exposure to animals in women exposed to food producing animals.
Methods: We used data and blood samples from the Danish National Birth Cohort. Serum samples collected during the first trimester from 192 pregnant women who were occupationally exposed to domestic animals and 188 randomly selected unexposed pregnant women were analysed for IgG, IgM, and IgA antibodies against Campylobacter, Salmonella, and Yersinia. Pregnancy outcomes of interest were identified through the Danish National Patient Register.
Results: Women with occupational exposure to animals had significantly higher IgG antibody concentrations against Campylobacter, Salmonella, and Yersinia, whereas they had lower concentrations of IgM and IgA antibodies.
Conclusions: Serological markers were not identified as risk factors for adverse pregnancy outcomes, with the exception of elevated concentrations of Salmonella antibodies, which were found to be associated with an increased risk of preterm birth.

1. Introduction

Campylobacter, Salmonella, and Yersinia are well known causes of bacterial gastroenteritis in humans. In industrialized countries, most infections are acquired by consuming or handling contaminated food. However, infections may also be transmitted from colonized animals,[1,2] and therefore professionals who come into occupational contact with domesticated animals may be at risk of exposure. In contrast to some other zoonoses that are known to cause adverse pregnancy outcomes (e.g. *Coxiella burnetii*, *Toxoplasma gondii*, and *Listeria monocytogenes*), little is known about the impact on pregnancy outcomes following infection with these zoonoses.

Campylobacter colonizes the intestine of various animals and is the most frequently reported gastrointestinal bacterial pathogen in humans in the European Union.[1] In humans, the species *Campylobacter jejuni* and *Campylobacter fetus* may also cause septic abortions, preterm birth, and sepsis in the mother.[3–6] Experiments on mice injected with Campylobacter at different stages of gestation have demonstrated an impaired implantation of embryos and foetal growth, as well as resorption of the foetus.[5]

Salmonella enterica can be categorized into at least 2500 serotypes, of which *Salmonella* Typhi and *Salmonella* Paratyphi are anthroponoses and the remaining non-typhoid types (NTS) are zoonotic.[7] Salmonella may cause septic abortions in goats, sheep, and cattle,[8] and miscarriage, postpartum sepsis, chorioamnionitis,

* Corresponding author. Tel.: +45 32683688.
E-mail address: bjk@ssi.dk (B. Kantsø).

http://dx.doi.org/10.1016/j.ijid.2014.06.021

bacteraemia with transplacental infection, septic abortions, neonatal sepsis, and meningitis in humans.[9,10]

Yersinia enterocolitica causes acute gastrointestinal infections which may be followed by erythema nodosum and reactive arthritis.[11–13] The pathogen is present in animals, especially pigs, and is known to cause miscarriages in production animals.[14,15]

Around the time of blood sample collection for our study, the prevalence of Salmonella was 6.5% in Danish broiler chickens and 3.7% in pigs, whereas the prevalence of Campylobacter was as high as 47.1% in broilers and 68.8% in pigs.[16] More recent Danish studies on the prevalence of zoonotic pathogens have reported Campylobacter to be the most frequent food-borne pathogen in Denmark.[2]

The aim of this study was to investigate the risk of elevated antibody titres against Campylobacter, Salmonella, and Yersinia in Denmark in women occupationally exposed to domestic animals compared to unexposed women sampled from a population-based cohort of pregnant women. Further, we sought to evaluate adverse pregnancy outcomes (miscarriage, preterm birth, and small for gestational age) in relation to occupational exposure to domestic animals.

2. Methods

2.1. Study participants

The Danish National Birth Cohort (DNBC), a nationwide cohort of 100 418 pregnant women and their offspring,[17] served as a basis for the sampling of the study population. Enrolment in the DNBC took place between 1996 and 2002. All Danish pregnant women were invited to join the study at their first antenatal visit to the general practitioner. Information on exposures before and during the early part of pregnancy was collected by means of a computer-assisted telephone interview scheduled to take place during gestational week 12. During the interviews, data were collected on reproductive history, age, smoking status, and contact with pet animals, and very detailed questions were asked regarding occupational exposure to different animals. The interviews were performed if the women were able to respond within four phone calls, and if they agreed to participate.

Among women who participated in the first interview and also provided a blood sample ($n = 95\ 000$), the population for the present study was defined as follows: self-reported occupational contact with animals and singleton pregnancy ($n = 192$; exposed) and randomly selected sample with no contact with animals ($n = 188$; non-exposed). One blood sample collected during gestational weeks 6 to 12 was analysed for antibodies against Campylobacter, Salmonella, and Yersinia. Women exposed through their occupation were veterinarians ($n = 116$) or worked on a farm with at least 40 dairy cattle ($n = 76$). A more comprehensive description is given by Nielsen et al.[18]

The DNBC was followed with respect to pregnancy outcome through register linkage to the Medical Birth Register in the Danish Patient Register. The following adverse pregnancy outcomes were studied: miscarriage, defined as a foetal loss before 154 days (22 gestational weeks) after the first day of the last self-reported menstrual period; preterm delivery, defined as delivery prior to 37 weeks of gestation; and small for gestational age (SGA), defined as a birth weight corresponding to −2 standard deviations below the specific gestational age according to the intrauterine weight standard suggested by Marsál.[19] Analyses of SGA were restricted to children born at ≥37 completed gestational weeks.

2.2. Detection of antibodies to enteric pathogens

Antibodies were detected by ELISA. ELISAs were performed fully automatically using a Tecan Freedom EVOLyzer (Tecan, Switzerland). Briefly, microtitre plates (Nunc-Immuno PolySorp F96, Roskilde, Denmark) were coated overnight at 4 °C in coating buffer (0.1 M sodium carbonate pH 9.6 containing 0.2% phenol red), followed by four washes in wash buffer (phosphate-buffered saline (PBS), pH 7.4 with 0.1% Tween 20); blocking was done with wash buffer (although for the Campylobacter ELISA, PBS + 5% Tween 20 was used).

Test, control, and reference sera were diluted 1:400 in dilution buffer (wash buffer + 0.2% phenol red) applied in duplicate and were incubated at room temperature, followed by four washes in wash buffer. Horseradish peroxidase-labelled rabbit antiserum to human IgG, IgM, or IgA (Dako, Glostrup, Denmark) was diluted 1:2500, 1:1000, and 1:500, respectively, in dilution buffer, and added to each well, followed by incubation at room temperature and four washes.

Tetramethylene benzidine substrate (TMB; Kem-En-Tec, Copenhagen, Denmark) was added, incubated at room temperature, and the reaction finally stopped by adding 1 M H_2SO_4. Optical density (OD) was read at 450 nm with background correction at 620 nm. Reference serum was used to adjust for day-to-day variation.

All serological analyses were performed in a certified laboratory at Statens Serum Institut, Denmark. Laboratory personnel were blinded to the exposure status.

The Campylobacter assay detects human serum antibodies against *C. jejuni* and *Campylobacter coli*. A combination of *C. jejuni* serotypes O:1,44 and O:53 in a 1:1 ratio was used.[20] The Salmonella assay detects human antibodies against *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium by a mix of serovar Enteritidis lipopolysaccharide (LPS) and serovar Typhimurium LPS.[21–23] The Yersinia assay detects human antibodies against *Y. enterocolitica* serogroup O:3. The ELISA uses LPS from *Y. enterocolitica* serogroup O:3 as the detecting antigen.

2.3. Serum antibody cut-off

The three ELISAs for Campylobacter, Salmonella, and Yersinia are used routinely in the laboratory at Statens Serum Institut for the identification of reactive arthritis following gastrointestinal infections. The cut-offs described in this section are used as indicators of reactive arthritis.

The Campylobacter cut-off was set from the analysis of sera from 200 Danish blood donors and sera from 90 Campylobacter culture-confirmed positive patients. The upper cut-off was set as the 95th percentile of the blood donor sera, whereas the lower cut-off (negative) was set at the 20th percentile of the culture-confirmed sera. The levels between positive and negative were defined as inconclusive. The cut-off values are as follows: IgG positive ≥1.90, inconclusive <1.90 to >1.00, negative ≤1.00; IgM positive ≥1.60, inconclusive <1.60 to >0.65, negative ≤0.65; and IgA positive ≥0.85, inconclusive <0.85 to >0.35, negative ≤0.35. Using these cut-offs the sensitivity of the Campylobacter ELISA is 78% for IgG, 78% for IgM, and 76% for IgA, and the combined sensitivity is 92%, whereas the specificity is 82% for IgG, 46% for IgM, and 68% for IgA, and the combined specificity is 90%.

The cut-off for the Salmonella analysis was determined from the analysis of sera from 100 Danish blood donors and sera from 100 Salmonella culture-confirmed positive patients. The upper cut-off was set as the 95th percentile of the blood donors sera, whereas the lower cut-off was set at the 10th percentile of culture-confirmed sera from patients. The cut-off values are as follows: IgG positive ≥0.45, inconclusive <0.45 to >0.15, negative ≤0.15; IgM positive ≥0.40, inconclusive <0.40 to >0.30, negative ≤0.30; and IgA positive ≥0.35, inconclusive <0.35 to >0.20, negative ≤0.20. Using these cut-offs the sensitivity of the Salmonella ELISA is 77% for IgG, 89% for IgM, and 82% for IgA, and the combined sensitivity is 95%. The assay has a clinical specificity of 93%.