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Relationship between *Escherichia coli* virulence factors and postpartum metritis in dairy cows

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ABSTRACT

The objectives of this study were to report the prevalence of *Escherichia coli* and *Trueperella pyogenes* in the uterus of postpartum dairy cows before the onset of postpartum metritis (PPM) and to quantify their association with subsequent occurrence of PPM, to quantify the association between the presence of genes encoding *E. coli* virulence factors (VF) and PPM, and to determine the accuracy of using early postpartum uterine bacteriology results (bacteria and VF) to identify cows at risk of PPM. A prospective cohort study was conducted on 3 commercial dairy farms. Uterine swabs were collected from 371 Holstein dairy cows (3 commercial herds) at 1 to 7 d in milk and submitted to the laboratory for identification of *E. coli*, *T. pyogenes*, and *E. coli* VF. A total of 40 VF were tested using the radioactive probe hybridization method. Postpartum metritis was defined as the presence of a fetid watery red-brown uterine discharge, associated with fever (rectal temperature >39.5°C), and systemic signs of illness (dullness, reduced appetite, and milk production). Surveillance of PPM was done by trained farmers blinded to laboratory results and cows were followed until 21 d in milk. Statistical analyses were conducted using 2 × 2 tables and mixed logistical regression models. Prevalences of *E. coli*, *T. pyogenes*, and PPM were 42, 34, and 15%, respectively. A total of 32 VF were found in *E. coli* isolates. Most prevalent VF were extraintestinal pathogenic genes such as *fimH* (89%), *hlyE* (87%), and *iss* (70%). Cows positive for intrauterine *E. coli* were 3.2 times more likely to have subsequent PPM compared with bacteriologically negative cows. Cows with VF *hra1* in their uterus were 2.7 times more likely to have PPM than cows positive for *E. coli* and negative for *hra1* and 5.9 times more likely than bacteriologically negative cows. Cows with VF *kpsMTII* in their uterus were 3.2 times more likely to have PPM than cows positive for *E. coli* and negative for *kpsMTII* and 6.2 times more likely than bacteriologically negative cows. Using *E. coli*, *hra1*, and *kpsMTII* as predictors for subsequent PPM, positive predictive values were 23, 31, and 42%, respectively, whereas the negative predictive values were 91, 80, and 78%, respectively. Overall, these results showed that *E. coli* and some VF were associated with PPM.

Key words: *Escherichia coli*, virulence factors, metritis, dairy cow

INTRODUCTION

A wide variety of bacterial species are present in the uterine lumen of dairy cows during early postpartum period (Jeon et al., 2015; Wagener et al., 2015). These include *Escherichia coli*, *Trueperella pyogenes*, streptococci, staphylococci, *Pseudomonas* spp., *Clostridium* spp., and various gram-negative anaerobes such as *Fusobacterium necrophorum* and *Prevotella melaninogenica* (Williams et al., 2005; Werner et al., 2012; Sens and Heuwieser, 2013; Wagener et al., 2014, 2015). The presence of these bacteria varies over time during the postpartum period and the overall population generally declines during the first 50 d after parturition (Sheldon et al., 2002). However, in a proportion of cows, some of these bacterial species persist in the uterine lumen and can favor the development of postpartum uterine diseases such as metritis and endometritis (Sheldon, 2004).

Postpartum metritis (**PPM**) is a severe inflammation of all layers of the uterus, (endometrium, submucosa, muscularis, and serosa) and is caused by a bacterial infection in the lumen of the uterus (Bondurant, 1999). The disease generally occurs during the first 21 d after parturition in dairy cattle and is defined by the presence of a fetid watery red-brown vaginal discharge with fever, body temperature being >39.5°C, and systemic signs of illness, such as dullness and anorexia (Drillich et al., 2001). Bacterial species frequently associated with the occurrence of PPM in dairy cows are *E. coli* and *T. pyogenes* (Sheldon et al., 2010; Werner et al.,

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2012; Sens and Heuwieser, 2013; Wagener et al., 2014), and obligate anaerobes such as *F. necrophorum* and *P. melaninogenicus* (Williams et al., 2005). The specific role of each of these bacterial species in the pathogenesis of PPM is still not well understood. However, it has been suggested that *E. coli* could play an important role in this process as it is frequently found in the uterus in early postpartum period, and its presence has been associated with the increased prevalence of other bacterial species (Dohmen et al., 2000; Williams et al., 2007; Bicalho et al., 2012) and the occurrence of severe uterine lesions and subsequent infertility (Sheldon et al., 2002; Williams et al., 2007).

Some recent studies focused on intrauterine *E. coli* and demonstrated that the genes encoding certain *E. coli* virulence factors (**VF**), such as *cdt*, *astA*, *ibeA*, *hlyA*, *hlyE*, *fyuA*, and *fimH*, are associated with PPM (Bicalho et al., 2010, 2012). It was suggested that the products of these genes may mediate induction of lesions in the uterine mucosa and promote the growth of opportunistic bacterial species, such as *T. pyogenes* and *F. necrophorum*, which would ultimately induce clinical signs of PPM (Bicalho et al., 2012). The disease was associated with negative effects in dairy cows, such as reduced milk production and lower reproductive performances (Toni et al., 2015; Piccardi et al., 2016).

Some studies have attempted to evaluate the beneficial effect of treating postpartum dairy cows to prevent PPM and avoid the impairment of productive and reproductive performances. Although Drillich et al. (2006) found that treating postpartum cows with metritis did not have a significant effect on milk production and reproductive performance, Risco and Hernandez (2003) revealed that treating postpartum cows affected by retained placenta significantly decreased the prevalence of subsequent PPM but did not improve subsequent reproductive performance. These results suggest that if a treatment is applied before the onset of clinical signs, PPM could potentially be prevented. Therefore, early identification of cows with high risk of developing PPM could help farmers to make a decision on whether or not to treat the cows. As *E. coli* colonizes the uterus immediately after parturition (Dohmen et al., 2000; Bicalho et al., 2012), and some *E. coli* VF have been associated with PPM (Bicalho et al., 2010, 2012), early identification of postpartum cows positive for intrauterine *E. coli* and positive for certain *E. coli* VF could inform on the risk level of cows for developing PPM.

Early postpartum uterine bacteriological analysis, including detection of *E. coli* VF, is readily available to veterinary practitioners and could be used for early identification of high-risk cows. Unfortunately, few data are available to allow the validation of such an approach. Therefore, the objectives of this study were (1) to report the prevalence of *E. coli* and *T. pyogenes* in the uterus of postpartum dairy cows before the onset of PPM and quantify their association with subsequent occurrence of PPM, (2) to quantify the association between *E. coli* VF and the subsequent development of PPM, and (3) to determine the accuracy of using early postpartum uterine bacteriology results (bacteria and VF) to identify cows at risk of PPM.

MATERIALS AND METHODS

Farm Selection and Sample Collection

A prospective cohort study was conducted from November 2011 to June 2012 on 3 commercial Holstein dairy farms. Convenient farm selection was used and based on motivation of the farmers to diagnose and report uterine diseases, as well as on convenience of being located within 30 km of the bovine ambulatory clinic of the Faculté de médecine vétérinaire, Université de Montréal (St-Hyacinthe, QC, Canada). Participating herds had computerized health event records, and were enrolled on a biweekly (every other week) herd health veterinary program and a monthly testing of dairy herd improvement program. All farms had freestall housing for cows.

Farms were visited weekly by a research technician and a veterinarian. All sampling was done by the same research technician. During farm visits, all cows between 1 and 7 d after parturition that did not yet show clinical signs of PPM were sampled by the research technician. An estimated sample size of 360 cows was targeted for the study. Sample size calculation was based on objective 2 to find a significant difference (error α: 5%; error β: 20%) in PPM prevalence between cows carrying *E. coli* positive for a specific VF (PPM prevalence: 35%) and those not carrying such *E. coli* (PPM prevalence: 15%), and expecting a prevalence of 20% for this VF in the studied population. Enrolled cows were sampled once during the study. For uterine sampling, cows were restrained and the perineum was cleaned and disinfected with 70% ethyl alcohol solution (Isopropylic Alcohol 70% USP; Green Field Inc., Brampton, ON, Canada). A sterile double-guarded uterine swab (Guarded culture swab; Jorvet Inc., Loveland, CO) was introduced in the vagina until it reached the body of the uterus. The swab was then exposed to the dorsal aspect of the uterine wall and pulled back inside its sheath. The swab was then placed in an anaerobic transportation medium (BBL Port-A-Cult Tubes; Becton, Dickinson and Company, Sparks, MD) and kept at 4°C until submission to the veterinary diagnostic laboratory of the Université de Montréal within 12 h of collection.

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