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Short communication: Diagnostic performance of on-farm bacteriological culture systems for identification of uterine *Escherichia coli* in postpartum dairy cows

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ABSTRACT

The objectives of this study were to validate the performance of on-farm bacteriological culture systems for identification of *Escherichia coli* in the uterus of early postpartum dairy cows and to determine if an association is present between the results and the subsequent occurrence of puerperal metritis (PM). A prospective cohort study was conducted in one commercial Holstein dairy herd in which 400 cows were sampled between 24 and 48 h after parturition. Three bacteriological samples were obtained from the uterus of each cow and were cultured for identification of *E. coli*. One sample was cultured in a commercial bacteriology laboratory according to standard procedures for identification of *E. coli* and was considered as the reference test. The two other samples were cultured on the farm using the Tri-Plate (University of Minnesota, St. Paul, MN) and Petrifilm systems, and plate readings were done after 24 and 48 h of incubation (variables: Tri24h, Tri48h, Petri24h, Petri48h). Participating cows were followed until 21 days in milk to diagnose PM. The prevalence of PM and *E. coli* (from the reference test) in the cow population was 15.0 and 33.5%, respectively. Both on-farm culture systems were accurate compared with the reference test. The sensitivity and specificity were 97 and 100%, 99 and 100%, 100 and 92%, and 100 and 89% for Tri24h, Tri48h, Petri24h, and Petri48h, respectively. On-farm results for Tri24h, Tri48h, Petri24h, and Petri48h were associated with subsequent occurrence of PM. The results from this study support the use of the Tri-Plate and Petrifilm culture systems on dairy farms to identify the presence of *E. coli* in the uterus of postpartum cows.

Key words: *Escherichia coli*, bacteriology, metritis, dairy cow

Short Communication

Various bacterial species can be found in the uterus of dairy cows during the early postpartum period (Jeon et al., 2015; Wagener et al., 2015). The presence of certain specific bacteria in the uterus of these cows, including *Escherichia coli* and some of its key virulence factors, was shown to increase the risk of puerperal metritis (**PM**; Sheldon et al., 2010; Bicalho et al., 2012; Kassé et al., 2016), which is defined as the presence of a fetid, watery, red-brown vaginal discharge with a fever (body temperature >39.5°C) and systemic signs of illness occurring within the first 21 DIM (Sheldon et al., 2006).

The benefit of treating postpartum dairy cows to prevent PM remains controversial. Although some studies showed that treating high-risk cows (generally affected by retained placenta, dystocia, twinning, or abortion) early after parturition decreased the subsequent occurrence of PM (Risco and Hernandez, 2003; McLaughlin et al., 2013), other studies did not report any benefit (Overton et al., 2003; Dubuc et al., 2011). Although the occurrence of PM is associated with risk factors such as retained placenta, dystocia, and abortion (Giuliodori et al., 2013), it could be speculated that these conditions are risk factors in part because they increase the amount of bacteria like *E. coli* in the uterus and thus can lead to PM. This hypothesis supports the fact that the presence of *E. coli* in the early postpartum period is an important risk factor for PM (Sheldon et al., 2010; Bicalho et al., 2012; Kassé et al., 2016). Currently, postpartum uterine samples can be processed in commercial bacteriology laboratories to identify the presence of *E. coli* using traditional culture tests. Assuming that early identification of cows with uterine *E. coli* could help prevent subsequent PM, it would be useful to develop or validate on-farm bacteriological culture systems for uterine samples. Although no data on this topic are available in the literature, such on-farm culture systems have been developed and validated for use in udder health management programs (McCarron et al., 2009; Royster et al., 2014). The same culture systems could

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be used for uterine samples, but this has not yet been assessed.

Therefore, the first objective of this study was to validate the performance of on-farm bacteriological culture systems to identify *E. coli* in the uterus of early postpartum dairy cows. The second objective was to determine if an association is present between these on-farm culture results and the subsequent occurrence of PM.

A prospective cohort study was conducted on one commercial Holstein dairy farm (250 lactating dairy cows) located in the vicinity of the bovine ambulatory clinic of the Faculté de Médecine Vétérinaire of the Université de Montréal (St-Hyacinthe, QC, Canada). The study was conducted between January 2015 and June 2016; cows were only allowed to be enrolled once in the study. Procedures of sample collection were approved by the animal care committee of the Université de Montréal. Farm selection was based on the farm's ease and ability of using on-farm bacteriology culturing systems for udder health management. The participating farm had a free stall barn and computerized records, and was enrolled in a veterinary herd health program every 2 wk. One full-time farm employee (a veterinarian) was in charge of data collection during the entire study. All fresh cows were systematically enrolled at parturition; the only exclusion criteria was for down cows at calving that were not included. All cows were examined and sampled between 24 and 48 h after calving. At 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). Three sterile double-guarded uterine swabs (guarded culture swab, Jorvet Inc., Loveland, CO) were introduced into the vagina until they reached the body of the uterus. The swabs were then exposed to the dorsal aspect of the uterine wall and pulled back inside their sheaths. One of the swabs was 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. At the laboratory, the swabs were plated on MacConkey agar (Oxoid, Ottawa, ON, Canada) and incubated at 37°C for 48 h for identification of gram-negative bacteria. All these bacteria were identified using triple sugar iron, urea, indole, and citrate tests. The presence and abundance of *E. coli* were reported on the laboratory results reports. The two other swabs were kept on the farm for bacteriological culture. One swab was cultured using the Tri-Plate culture system (University of Minnesota, St. Paul, MN) and the other one was cultured using the coliform Petrifilm culture system (coliform count plate, 3M, London, ON, Canada). The procedures for these on-farm cultures were similar to the ones followed for udder health management (McCarron et al., 2009; Royster et al., 2014). In summary, one swab was smeared on the surface of a Tri-Plate, and this plate was then incubated at 37°C in an egg incubator (Hovabator 1602N, GQF Manufacturing, Savannah, GA) for 48 h. For the Petrifilm system, the swab was plunged into 1 mL of sterile water left in a sterile glass vial and shaken for 30 s. Subsequently, 1 mL of that solution was gently dropped on the Petrifilm surface and the plate was incubated at 37°C in the same egg incubator for 48 h. Both the Tri-Plate and Petrifilm plates were read after 24 and 48 h of incubation.

Sample size calculation was based on two calculations. For the first objective of the study, a sample size of 240 cows was estimated based on expecting a sensitivity of 80% and specificity of 80% for the on-farm tests, a minimal acceptable lower confidence limit of 60%, and an expected prevalence of *E. coli* in uterine samples of 25% (Flahault et al., 2005). For the second objective of the study, a sample size of 400 cows was estimated to find a significant difference (error alpha: 5%; error beta: 20%) in PM prevalence between cows carrying *E. coli* (PM prevalence: 25%) and those not carrying *E. coli* (PM prevalence: 5%), and expecting a prevalence of 25% for *E. coli* in the studied population (Dohoo et al., 2009). Therefore, a total of 400 cows were targeted for the study.

Before the start of the study and every month during data collection, the definition of PM was reviewed and standardized with the participating farm staff. All cases of PM were confirmed by the farm staff manager (veterinarian) in charge of the project and of data collection. Puerperal metritis was defined as the presence of a fetid, watery, red-brown uterine discharge, associated with a fever (rectal temperature >39.5°C) and systemic signs of illness (dullness, and reduced appetite and milk production) within the first 21 d after parturition (Sheldon et al., 2006). Farm staff were blinded to laboratory results. When PM was diagnosed, cows were treated based on their usual PM treatment protocol, which was 5 d of ceftiofur administered i.m. once a day (2.2 mg/kg; Zoetis animal health, Kirkland, QC, Canada). When a case of PM was diagnosed, it was noted in the computerized records.

Statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., Cary, NC). The experimental unit of the study was the cow. Individual cow data, laboratory results, and on-farm bacteriology results were combined into a spreadsheet. Cows were considered positive for *E. coli* if the laboratory reported that at least 10 colonies were isolated from the swabs after 48 h of incubation, and this was considered the