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

J. Dubuc¹

Département de Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, St-Hyacinthe, QC, J2S 7C6 Canada

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

Received November 15, 2016.

Accepted January 1, 2017.

¹Corresponding author: jocelyn.dubuc@umontreal.ca

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 (isopropyl 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

Table 1. Test performance of different on-farm bacteriology culture systems to identify *Escherichia coli* in postpartum uterine samples from dairy cows compared with standard commercial laboratory results

Test ¹	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Kappa
Tri-Plates (24 h)	97.0	100	100	98.5	0.85
Tri-Plates (48 h)	99.3	100	100	99.6	0.88
Petrifilms (24 h)	100	92.4	87.0	100	0.81
Petrifilms (48 h)	100	88.7	81.7	100	0.83

¹Tri-Plates (University of Minnesota, St. Paul, MN); Petrifilms (3M, London, ON, Canada).

reference test. For Tri-Plates, cows were considered positive if at least 10 colonies were isolated from the swabs. Two variables were created: one for the plate reading after 24 h of incubation (**Tri24h**) and one after 48 h (**Tri48h**). For Petrifilms, cows were considered positive if at least 10 colonies were isolated from 1-mL solution samples. Similar to Tri-Plates, 2 variables were created for plate readings after 24 h (**Petri24h**) and 48 h (**Petri48h**).

Descriptive statistics were calculated using Proc FREQ in SAS. For the first objective of the study, 2 × 2 tables were built to compare individual Tri-Plate and Petrifilm results with the reference test results (Proc FREQ). Using these tables, sensitivity (**Se**), specificity (**Sp**), positive predictive value (**PPV**), and negative predictive value (**NPV**) were calculated for Tri24h, Tri48h, Petri24h, and Petri48h. Agreement beyond chances (kappa) was also computed. For the second objective of the study, logistic regression models were built using PM as the dependent variable and parity and season of calving as confounders (Proc GLIMMIX). One model was built for each predictor: laboratory result for *E. coli*, Tri24h, Tri48h, Petri24h, and Petri48h. All of these models were compared using their Akaike information criteria (**AIC**), and the lowest value was desirable (Burnham and Anderson, 1998). The AIC value can be used to compare nonnested models, and better models provide lower AIC values than worse models (Burnham and Anderson, 1998; Dohoo et al., 2009).

A total of 400 cows were sampled in the present study. Samples were collected from 140 primiparous cows (35%) and 260 multiparous cows (65%). The prevalence

of PM in the study was 15% (n = 60). The average time after parturition at which PM was diagnosed was 7 (SD ± 3) d and ranged from 3 to 20 d. Based on laboratory results (reference test), the prevalence of *E. coli* was 33.5% (n = 134), whereas it was 32.5% (n = 130), 33.3% (n = 133), 38.5% (n = 154), and 41.0% (n = 164) for Tri24h, Tri48h, Petri24h, and Petri48h, respectively. Table 1 presents the Se, Sp, PPV, NPV, and kappa of Tri24h, Tri48h, Petri24h, and Petri48h. Table 2 presents the prevalence of PM stratified by different tests results used (reference test, Tri24h, Tri48h, Petri24h, and Petri48h) for identifying the presence of *E. coli*. Accounting for parity and season of calving, the laboratory results were associated with PM [odds ratio (**OR**): 2.9; 95% CI: 1.8–4.0; *P* = 0.01], and its AIC value was 254.8. In similar models, Tri24h (OR: 2.2; 95% CI: 1.2–3.3; *P* = 0.02), Tri48h (OR: 2.9; 95% CI: 1.9–4.0; *P* = 0.01), Petri24h (OR: 2.2; 95% CI: 1.2–3.2; *P* = 0.02), and Petri48h (OR: 1.4; 95% CI: 1.1–1.9; *P* = 0.02) were also associated with PM, and their AIC values were 258.3, 255.9, 269.1, and 265.4, respectively.

The present study is one of the first to describe the diagnostic accuracy of on-farm bacteriology culture systems for identification of *E. coli* in uterine samples from postpartum dairy cows. These results showed that both Tri-Plate and Petrifilm culture systems could be used on farms because they are accurate compared with the reference test (traditional bacteriology laboratory test). The logistic regression models also showed that the results obtained from the laboratory and from the on-farm culture systems were associated with subsequent occurrence of PM. If one of these 2 systems had to be chosen for on-farm use, the Tri-Plate system

Table 2. Prevalence of puerperal metritis (PM) stratified by different bacteriological test results for the presence of *Escherichia coli* in uterine samples from postpartum dairy cows

Test ¹	PM prevalence when <i>E. coli</i> was not found (%)	PM prevalence when <i>E. coli</i> was found (%)
Laboratory-reference test	10.2	24.6
Tri-Plates (24 h)	11.5	22.3
Tri-Plates (48 h)	10.1	24.8
Petrifilms (24 h)	11.0	21.4
Petrifilms (48 h)	15.7	20.1

¹Tri-Plates (University of Minnesota, St. Paul, MN); Petrifilms (3M, London, ON, Canada).

might be the most appropriate system because the sums of its Se and Sp were higher than those from the Petrifilm system. This finding means that there would be less misclassification bias with Tri-Plates, and this is supported by the fact that the AIC values for the Tri-Plates logistic regression models were lower than those for Petrifilms. Regarding which delay is best for reading these culture system results, it appears that 48 h provides the greatest accuracy and the lowest AIC values for both systems. If a user's goal is to quantify the prevalence of *E. coli* on farms as accurately as possible using these systems, it needs to be kept in mind that, based on these results, Tri-Plates would slightly underestimate this value, whereas Petrifilms would slightly overestimate it. However, the optimal system would be the use of Tri-Plates and to read those 48 h after incubation. If an antibiotic were to be used on positive cows to prevent PM, these factors affecting the prevalence should be considered to decide if it is better to over- or undertreat cows.

Based on the current literature, it remains unclear if traditional laboratory bacterial culture is really the reference test for identifying the presence of *E. coli* in the uterus of postpartum dairy cows. It was suggested that other diagnostic methods such as PCR tests could lead to different results (Jeon et al., 2015; Wagener et al., 2015). In such case, the current study provides data to show that the agreement between laboratory results and these on-farm culture system is almost perfect (Dohoo et al., 2009), although the kappa values are higher for the Tri-Plates than for the Petrifilms. It thus suggests that if one was looking for an alternative test to the traditional laboratory bacterial culture, Tri-Plates would provide results that are similar to the laboratory results than the Petrifilms. Future studies should explore the possibility that there is no reference test in this case and the use of Bayesian statistical analyses could be considered to evaluate the test performance as suggested by De Boer et al. (2014).

Overall, these results support the use of Tri-Plates and Petrifilms as on-farm bacteriology culture systems for uterine samples from postpartum dairy cows, because they are accurate tests compared with the laboratory culture system and their results are associated with subsequent occurrence of PM.

ACKNOWLEDGMENTS

This research project was supported by the Zoetis clinical research fund from the bovine ambulatory clinic of the Faculté de Médecine Vétérinaire of the Université de Montréal (St-Hyacinthe, QC, Canada). The author acknowledges the participating farm and its devoted

staff for its willingness to innovate and perform data collection.

REFERENCES

- Bicalho, M. L. S., V. S. Machado, G. Oikonomou, R. O. Gilbert, and R. C. Bicalho. 2012. Association between virulence factors of *Escherichia coli*, *Fusobacterium necrophorum*, and *Arcanobacterium pyogenes* and uterine diseases of dairy cows. *Vet. Microbiol.* 157:125–131.
- Burnham, K. P., and D. R. Anderson. 1998. *Model Selection and Multimodel Inference*. 2nd ed. Springer, New York, NY.
- de Boer, M. W., S. J. LeBlanc, J. Dubuc, S. Meier, W. Heuwieser, S. Arlt, R. O. Gilbert, and S. McDougall. 2014. Invited review: Systematic review of diagnostic tests for reproductive-tract infection and inflammation in dairy cows. *J. Dairy Sci.* 97:3983–3999.
- Dohoo, I. R., S. W. Martin, and H. Stryhn. 2009. *Veterinary Epidemiologic Research*. 2nd ed. VER Inc., Charlottetown, P.E.I., Canada.
- Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2011. Randomized clinical trial of antibiotic and prostaglandin treatments for uterine health and reproductive performance in dairy cows. *J. Dairy Sci.* 94:1325–1338.
- Flahault, A., M. Cadilhac, and G. Thomas. 2005. Sample size calculation should be performed for design accuracy in diagnostic test studies. *J. Clin. Epidemiol.* 58:859–862.
- Giuliodori, M. J., R. P. Magnasco, D. Becu-Villalobos, I. M. Lacau-Mengido, C. A. Risco, and R. L. de la Sota. 2013. Metritis in dairy cows: Risk factors and reproductive performance. *J. Dairy Sci.* 96:3621–3631.
- Jeon, S. J., A. Vieira-Neto, M. Gobikrusheranth, R. Daetz, R. D. Mingoti, A. C. Parize, S. L. de Freitas, A. N. da Costa, R. C. Bicalho, S. Lima, K. C. Jeong, and K. N. Galvao. 2015. Uterine microbiota progression from calving until establishment of metritis in dairy cows. *Appl. Environ. Microbiol.* 81:6324–6332.
- Kassé, F. N., J. M. Fairbrother, and J. Dubuc. 2016. Relationship between *Escherichia coli* virulence factors and postpartum metritis in dairy cows. *J. Dairy Sci.* 99:4656–4667.
- McCarron, J. L., G. P. Keefe, S. L. B. McKenna, I. R. Dohoo, and D. E. Poole. 2009. Laboratory evaluation of 3M Petrifilms and University of Minnesota Bi-plates as potential on-farm tests for clinical mastitis. *J. Dairy Sci.* 92:2297–2305.
- McLaughlin, C. L., E. P. Stanisiewski, C. A. Risco, J. E. P. Santos, G. E. Dahl, R. C. Chebel, C. LaGrow, C. Daugherty, L. Bryson, D. Weigel, J. Hallberg, and M. J. Lucas. 2013. Evaluation of ceftiofur crystalline free acid sterile suspension for control of metritis in high-risk lactating dairy cows. *Theriogenology* 79:725–734.
- Overton, M. W., W. M. Sischo, and J. P. Reynolds. 2003. Evaluation of effect of estradiol cypionate administered prophylactically to postparturient dairy cows at high risk for metritis. *J. Am. Vet. Med. Assoc.* 223:846–851.
- Risco, C. A., and J. Hernandez. 2003. Comparison of ceftiofur hydrochloride and estradiol cypionate for metritis prevention and reproductive performance in dairy cows affected with retained fetal membranes. *Theriogenology* 60:47–58.
- Royster, E., S. Godden, D. Goulart, A. Dahlke, P. Rapnicki, and J. Timmerman. 2014. Evaluation of the Minnesota Easy Culture System II Bi-plate and Tri-Plate for identification of common mastitis pathogens in milk. *J. Dairy Sci.* 97:3648–3659.
- Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. *Theriogenology* 65:1516–1530.
- Sheldon, I. M., A. N. Rycroft, B. Dogan, M. Craven, J. J. Bromfield, A. Chandler, M. H. Roberts, S. B. Price, R. O. Gilbert, and K. W. Simpson. 2010. Specific strains of *Escherichia coli* are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease in cattle and mice. *PLoS One* 5:e9192.
- Wagener, K., I. Prunner, H. Pothmann, M. Drillich, and M. Ehling-Schulz. 2015. Diversity and health status specific fluctuations of intrauterine microbial communities in postpartum dairy cows. *Vet. Microbiol.* 175:286–293.