

REGULAR ARTICLE

Agreement among bacteriological findings, vaginal discharges, and endometrial cytology for endometritis detection in postpartum beef cows

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ABSTRACT

Aim of the study: This study aims to demonstrate the relationship among vaginal secretions, bacterial isolates, and endometrial cytology during 20–30 days postpartum in beef cows. **Background:** Endometritis is one of the most common diseases that occurs in dairy cow after several weeks postpartum period. There is two types of endometritis, clinical and subclinical endometritis that cause severe economic losses by increased days open, calving interval and numbers of services to conception. **Materials and Methods:** A total of 82 beef cows, aged 3 years to 7 years and at 20–30 days post-calving, were enrolled in this study. All the cows were checked by transrectal palpation, ultrasound, and vaginal secretions collection. A four-grade system (0 = clear mucus, 1 = mucus containing flecks of pus, 2 = discharge including < 50% pus, and 3 = involving > 50% pus) was used to categorize vaginal secretions of these cows. Endometrial cytology and bacteriological samples were then collected using a cytobrush technique. **Results:** Nine of 82 beef cows (11%) had abnormal vaginal secretions (grade 1-3) and indicated to clinical endometritis (CE), and nine of 73 clinically healthy cows (12.32%) had subclinical endometritis (SCE, $\geq 8\%$ PMN cows). The agreement among endometrial examination, polymorphonuclear neutrophil percentage (PMN %), and vaginal discharge score (0–3) was moderate ($k = 0.48$, $p < 0.01$), whereas that between vaginal discharge score (0–3) and bacteriological finding was poor ($k = 0.032$, $p = 0.51$). The agreement between bacterial finding and PMN % was also poor ($k = 0.15$, $p = 0.02$). *Escherichia coli* was the most common bacteria isolated from healthy (14.6%), CE (38.8%), and SCE (42.8%) cows. Moreover, *E. coli* was the major bacteriological risk factor for SCE occurrence. *E. coli* and *Staphylococcus aureus* were the most common risk factors for the occurrence of CE. **Conclusion:** A moderate agreement exists among PMN %, bacteriological findings, and vaginal secretions, whereas a poor agreement exists between bacterial findings and PMN %. *E. coli*, *S. aureus*, and dystocia were the major factors affecting uterine infection in beef cows. CE and SCE insignificantly affected the reproductive performance of beef cows.

Keywords: Beef cows; Endometrial cytology; Endometritis; Postpartum uterus; Risk factors

INTRODUCTION

All postpartum cows suffer from pathogenic microorganism contamination of the uterus 2 weeks to 3 weeks after parturition, and more than 80% of the cows after calving are exposed to uterine contamination by *Escherichia coli*, *Fusobacterium* sp., *Arcanobacterium pyogenes*, *Streptococcus* sp., *Staphylococcus* sp., *Clostridium* sp., *Pasteurella multocida*, and *Bacteroides* sp (Sheldon et al., 2006). McDougall et al. (2011) isolated and registered more than 35 bacteria species that increased the risk for genital tract infection after parturition in cows.

Uterine infection can be categorized into puerperal metritis, clinical metritis, clinical endometritis, and subclinical endometritis (Sheldon et al., 2006). Clinical endometritis (CE) is known as endometrial inflammation with purulent or mucopurulent discharge; this disease can be detected 21 days after calving and is not associated with clinical signs of sickness (Sheldon et al., 2006). The correct diagnosis of CE depends on the presence of vaginal discharge (LeBlanc et al., 2002). CE can be diagnosed using a vaginoscope, meter check device, or gloved hand (Leutert et al., 2012). The term “purulent vaginal discharge” has been adopted for use as instead of term CE because the

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presence of abnormal genital discharge does not necessarily indicate endometrial inflammation (Dubuc *et al.*, 2010). Subclinical endometritis (SCE) is known as the endometrial inflammation of the uterus without mucopurulent material accumulation in the vagina (Sheldon *et al.*, 2006). SCE has also been identified as cytological endometritis (Gilbert *et al.*, 2005). Dubuc *et al.* (2010) described cytological endometritis as an elevated ratio of polymorphonuclear neutrophils (PMN) in endometrial cytology samples obtained by cytobrush or low-volume uterine lavage. Assessment of cows suffering from endometrial infections is hampered by the lack of consensus on an acceptable definition of endometritis in cows (Gilbert *et al.*, 2005; Sheldon *et al.*, 2006) and simple, effective diagnostic methods. Despite that transrectal palpation of the uterus is commonly used to diagnose endometritis, diagnosis, this technique cannot accurately identify cows with endometritis and subsequent reduced fertility (Runciman *et al.*, 2008).

SCE can be detected using several methods, some of which can be performed during collection of endometrial cells. In these techniques, endometrial and inflammatory cells may be collected using a guarded cotton swab (Studer and Morrow, 1978), uterine biopsy (Bourke *et al.*, 1997), uterine lavage (Gilbert *et al.*, 2005), or cytobrush (Kasimanickam *et al.*, 2004). However, the significant challenge in SCE diagnosis is the absence of consensus among studies regarding the sampling time and cutoff values to differentiate diseased from healthy cows. Scholars have suggested many threshold values for the proportion of PMN, ranging from 10% to 18% (Kasimanickam *et al.*, 2004).

The occurrence of uterine diseases in beef cows in Malaysia remains unclear, and the relationship between bacterial contamination and rate of postpartum uterine diseases has been rarely investigated. The present study aims to assess the relationship among bacterial contamination, vaginal discharges, and endometrial cytology findings in postpartum beef cows and to determine the diagnostic value of these parameters for detection of CE and SCE.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (Ref. UPM/IACUC/AUP-R099/2015; 10 February 2016)

One hundred cows (55 Brangus and 45 Kedah-Kelantan breeds) at 20 days to 30 days post-calving period were obtained from three different private beef farms and all samples were collected from animals while cows remained at their farm of origin between June 2015 to October

2016. The beef farms are located in Serdang, Selangor where the temperature is 28 °C, and relative humidity is about 70%. The cows were aged 3–7 years, weighed 300–450 kg, and managed under free grazing. The cows were also fed according to field management after providing concentrated feed, which consisted of alfalfa, corn silage, beet pulp, cottonseed, soybean, corn, and barley. Individual animal data on calving history, lactation, breed, parity, and so on were recorded. The farms used many bulls with a high fertility and passed a breeding soundness examination every two month and these farms used 20:1 as cow-to-bull ratio for natural mating after the postpartum period. The body condition score (BCS) of the cows was evaluated by using a 5-point scale (Ferguson *et al.*, 1994). Pregnancy diagnosis for cows was achieved by using ultrasound technique at 150 and 200 days after calving.

Examination of cows

All cows were checked by rectal palpation 20 to 30 days after calving to evaluate degree of uterine involution, symmetry of uterine horns, and position of the uterus relative to the pelvic rim. Genital tract discharge of all cows was checked through examination of vaginal secretions by using hands covered with clean disposable long gloves at 20 days to 30 days post-calving to identify CE cases. A four-grade system (0 = clear mucus, 1 = mucus containing flecks of pus, 2 = discharge including < 50% pus, and 3 = involving > 50% pus) was used to categorize vaginal secretions (Williams *et al.*, 2005). The vulva and perineum region were washed, cleaned, sterilized with iodine, and dried using clean, sterile paper towels. The area was then lubricated (Triad Sterile Lubricating Jelly, H&P Industries Inc., Mukwonago, WI, USA). The hand covered with a long sterile disposable glove was inserted into the vagina far enough to allow observation of the nature of the fluid; if necessary, a light source was used to obtain evidence of abnormal cervical secretion (Barlund *et al.*, 2008).

Endometrial cytology using cytobrush method

Endometrial cytological samples were collected from cows by using a modified sterile Cytobrush Plus GT (Medscand Medical, Germany) (Madoz *et al.*, 2013). The handle was shortened to 2 cm and made thread-like to enable insertion into a stainless steel rod (artificial insemination AI gun 65 cm in length, 4 mm outside diameter). The cytobrush and stainless steel rod combination were protected by a plastic sheath (Chemise Sanitaire, IMV Technologies, France) to avoid vaginal contamination, lubricated (Triad Sterile Lubricating Jelly, H&P Industries Inc., Mukwonago, WI, USA), and introduced into the vagina. A sleeved arm was introduced into the rectum to facilitate passage of instruments through the genital tract and os cervix. When the instrument reaches the uterus, the cytobrush was exposed and turned many times to

collect cellular material from the adjacent endometrium. The cytobrush was again protected by the plastic sheath and withdrawn from the genital tract. The sample was rolled on the sterile microscopic slide (75mm×25mm) and stored in a transport medium (LABCHEM SDN.BHD) for bacteriological analysis. All slides were fixed with methanol for 30 min, transported to the laboratory within 3 hours, stained with 5% Giemsa stain for 3 min, and dried. All of the slides were evaluated by counting 300 cells at 400× magnification (Leitz Laboulux-S, Wetzlar, Germany) to determine the percentage of neutrophils (PMN %). Endometrial threshold value $\geq 8\%$ was used to determine the SCE occurrence in the farms between 20 and 30 days postpartum (Madoz et al., 2013).

Bacteriological samples

Bacteriological samples were transferred into sterile tubes containing thioglycolate broth as transport medium, transported to the laboratory at 4 °C, and immediately processed for bacteriological examination. Samples were cultured aerobically on sheep blood agar, MacConkey agar, and nutrient agar. Bacterial growth on the culture plates was scored semi-quantitatively after 24 h of incubation at 37 °C for aerobic growth. Bacteria were identified based on the shape of the bacterial colony, Gram stain, hemolysis, morphology, and tests on coagulase, oxidase, catalase, indole production, and methyl red.

Experimental animals

One hundred cows enrolled in this study, 18 cows were excluded because of the presence of clinical mastitis (5 cows), sold (7 cows), lameness (3 cows) and inadequate endometrial cytological sampling (3 cows). The remaining 82 cows were identified as Brangus (54.87%, 45/82), Kedah–Kelantan (45.12%, 37/82), primiparous (29.2%, 24/82), and multiparous (70.8%, 58/82). Calving assistance (dystocia), retained placenta, and twins were recorded in 11% (9/82), 4.8% (4/82), and 2.4% (2/82) of the cows.

Data and statistical analyses

Clinical findings and all data were recorded and encoded into Excel software (Excel 2007, Microsoft Office Corporation, Redmond, WA, USA). The data included BCS, parity, date of parturition, calving assistance rendered, postpartum diseases, vaginal clinical findings (discharge score), cytological examination findings, bacteriological isolation results, and reproductive performance of cows. All the statistical methods were performed by SPSS software (version 18.0, IBM SPSS Inc., Chicago: USA) and Excel 2007. The occurrence of SCE and CE was determined based on clinical examination results, vaginal discharges, and endometrial cytological samples. The agreement among vaginal discharge grades, the percentage of PMN, and bacteriological findings were examined using

Kappa analyses (Santos et al., 2009). Chi-square analysis was used to compare the bacteriological results of healthy and infected cows. Moreover, the relative risk factor for the occurrence of SCE and CE was measured, considering isolated bacteria (*E. coli*, *Staphylococcus*, and *Bacillus*) consequently, just isolated bacteria with P value < 0.10 were considered to have significant effects and included in regression model. A binary logistic regression model was used to analyze factors for the occurrence of SCE and CE. These factors included infection with *E. coli* (0 = no infection, 1 = infection), infection with *Bacillus* spp. (0 = no infection, 1 = infection), infection with *Staphylococcus* spp. (0 = no infection, 1 = infection), calving (0 = normal, 1 = dystocia), and BCS (0 = ≥ 3 , 1 = > 3), parity (0 = primiparous, 1 = multiparous).

Reproductive performance parameters were reported by calculating the percentage of pregnancy rate at 150 and 200 days postpartum by using ultrasound technique. A Cox regression model for non-pregnancy within 200 days post-calving was established using the following grading system: CE (0 = CE, 1 = no CE), SCE (0 = SCE, 1 = no SCE), infection with *E. coli* (0 = no infection, 1 = infection), infection with *Bacillus* spp. (0 = no infection, 1 = infection), infection with *Staphylococcus* spp. (0 = no infection, 1 = infection), calving (0 = normal, 1 = dystocia), and BCS (0 = ≥ 3 , 1 = > 3) (Madoz et al., 2013). The level of significance was set at 0.05 for all statistical analyses, and the CI was set at 95%

RESULTS

Vaginal examination

Vaginal examination on postpartum days 20-30 showed nine cows (11%) with abnormal vaginal secretions. Four cows had a vaginal secretion score of 1, four cows were scored 2, and one cow was scored 3.

Bacterial isolation

Regarding bacterial isolates, twenty different microorganisms were identified among the 114 bacterial isolates obtained from 56 of the 82 (68%) postpartum cows; no bacterial isolates were obtained from the remaining 26 cows. Multiple bacterial species were isolated from 38 of the 56 cows (68%), whereas a single bacterial species was isolated from the remaining 18 cows. Bacterial isolates are shown in Table 1. *E. coli*, *Bacillus* sp., *Staphylococcus* sp., and *Streptococcus* sp. accounted for 21.9 % (25/114), 12.28% (14/114), 6.14% (7/114), and 13.15 % (15/114) of the bacteria species isolated, respectively (Table 1). *E. coli* was the most isolated bacteria from SCE (42.85%; 6/14), and CE cows (38.88%; 7/18), which was significantly higher ($p > 0.05$) than in healthy cows (14.6%; 12/82).

Table 1: Percentage of isolated bacteria in healthy, CE, and SCE cows

Species of bacteria isolated	Healthy cows 82 isolated bact (%)	CE* cows 18 isolated bact (%)	SCE** cows 14 isolated bact (%)
<i>Escherichia coli</i>	12 (14.63) ^a	7 (38.88) ^b	6 (42.85) ^b
<i>Bacillus spp.</i>	10 (12.19)	2 (11.11)	2 (14.28)
<i>Staphylococcus schleiferi</i>	2 (2.43)		1 (7.14)
<i>Staphylococcus aureus</i>	1 (1.21) ^c	4 (22.22) ^d	
<i>Streptococcus bovis</i>	3 (3.65)	1 (5.5)	1 (7.14)
<i>Streptococcus pneumonia</i>	4 (4.87)	-	1 (7.14)
<i>Streptococcus agalactia</i>	2 (2.43)	-	-
<i>Streptococcus zooepidemicus</i>	3 (3.65)		-
<i>A. pyogenes</i>	1 (1.21)	1 (5.5)	-
<i>Proteus spp.</i>	1 (5.5)	1 (5.5)	-
<i>Enterobact faecium</i>	4 (4.87)	1 (5.5)	-
<i>Alcaligenes faecalis</i>	5 (6.09)	-	-
<i>Enterobacter cloacae</i>	5 (6.09)	-	1 (7.14)
<i>Klebsiella pneumoniae</i>	3 (3.65)	1 (5.5)	1 (7.14)
<i>Acinetobacter baumannii</i>	4 (4.87)	-	1 (7.14)
<i>Acinetobacter Iwoffii</i>	4 (4.87)	-	-
<i>Acinetobacte rcalcoaceticus</i>	5 (6.09)	-	-
<i>Chromobacterium</i>	4 (4.87)	-	-
<i>Pantoea agglomerans</i>	4 (4.87)	-	-
<i>Pasteurella spp.</i>	2 (2.43)	-	-
Total bacterial isolation	82 (100)	18 (100)	14 (100)

*CE: Clinical endometritis, **SCE: Subclinical endometritis^a ^b ^c ^dDifferent lowercase letters in the same row indicate significant differences (p<0.05)

The commonly isolated bacteria from CE cows were *E. coli* (38.88%), *Staphylococcus aureus* (22.2%), *Bacillus sp.* (11.11%), *Streptococcus bovis* (5.5%), *Proteus sp.* (5.5.1%), and *A. pyogenes* (5.5. %). *Staphylococcus aureus* percentage was higher (p >0.05) in CE (22.2 %) than in healthy cows (1.21 %). The most common bacterial species present in SCE cows were *E. coli* (42.85%) and *Bacillus sp.* (14.2 %). Moreover, no bacteria were isolated from two cows (2/9) with SCE.

Endometrial cytology

All cows were sampled by cytobrush, of which, nine cows manifested SCE (12.3%, determined using $\geq 8\%$ PMN as threshold value and vaginal discharge score = 0). The average PMN % on the slides ranged from 0 to 29%. The agreement among endometrial examination, number of PMN (%), and vaginal discharge score (0–3) was moderate (k = 0.48, p => 0.01; Table 2). There is a poor agreement between vaginal discharge score (0–3) and bacteriological finding (k = 0.032, p = 0.51) and between bacterial finding and PMN (k = 0.15, p = 0.02) (Table 2).

Table 2: Beef cows with <8% and $\geq 8\%$ of polymorphonuclear leukocytes (PMN) in endometrial cytology samples, negative (score 0) or positive (scores 1, 2, and 3) gross vaginal inflammation score, and the presence or absence of bacterial contamination at days 20–30 post calving the Kappa statistic (K) is a measure of the level of agreement between the tests, where 1=complete agreement and 0=no agreement

	Bacterial isolation		Kappa
	Positive	Negative	
Cytobrush			
$\geq 8\%$ PMN	16	2	K=0.15
> 8%PMN	40	24	P=0.028
Vaginal discharges			
	Positive	Negative	
Bacterial isolation			
Positive	7	49	K=0.032
Negative	2	24	P=0.51
Vaginal discharges			
	Positive	Positive	
Cytobrush			
$\geq 8\%$ PMN	7	9	K=0.48
> 8%PMN	2	64	P=000

Risk factors affecting the occurrence of CE and SCE

E. coli was the major bacteriological risk factor for the occurrence of SCE (odds ratio (OR) = 8.6; 95% CL = 1.89–39.68; P = 0.03) and CE (OR = 7; 95% CL = 1.05–47.1; P = 0.04)(Table 2 and 3). *Staphylococcus aureus* was the major bacteriological risk factor for the occurrence of CE (OR = 10.16; 95% CL = 1.66–61.91; p = 0.01). Dystocia was considered a risk factor for CE occurrence in beef cows (OR = 10.6; 95% CL = 1.48–76.09; p = 0.02), whereas BSC was a risk factor only for SCE occurrence (OR = 11.7; 95% CL = 1.24–113.32; p = 0.01). Other isolated bacterial species and parity did not have obvious risk effects on the occurrence of CE and SCE (Table 3 and 4).

Reproductive performance

Cox regression model analysis showed that SCE, CE, factors, and bacterial agents did not significantly affect the risk for non-pregnancy at 200 days after calving (Table 5). The pregnancy rates at 150 and 200 days postpartum were 35.3% (29/82) and 45.12% (37/82), respectively. The percentages of pregnant cows at 150 days postpartum were 37.5% (24/64), 33.3% (3/9), and 22.2% (2/9) in healthy, SCE, and CE groups, respectively. The percentages of pregnant cows at 200 days postpartum were 46.8% (30/64), 44.4% (4/9), and 33.3% (3/9) in healthy, SCE, and CE groups, respectively (Table 6).

DISCUSSION

Beef cows consider a primary source of cattle for the meat industry and many problems are affecting the productivity

Table 3: Bacteriologic relative risk factors for cows diagnoses with clinical endometritis and subclinical endometritis between 20 and 30 days postpartum

Factor	Relative risk	95% CI	P value
Diagnosis CE*			
<i>Bacillus</i>	1.3	0.314-5.73	0.64
<i>E coli</i>	9.9	1.33-26.66	0.007
<i>Streptococcus sp.</i>	2.1	0.35-13.08	0.43
<i>Staphylococcus aureus</i>	8.9	3.2-24.6	0.01
<i>Trueperella pyogenes</i>	4.4	0.959-20.53	0.11
Diagnosis SCE**			
<i>E coli</i>	6.1	1.70-21.97	0.002
<i>Bacillus</i>	1.4	0.34-6.15	0.61
<i>Streptococcus sp.</i>	1.2	0.28-5.08	0.80

*CE: Clinical endometritis, **SCE: Subclinical endometritis

Table 4: Results of binary logistic regression analysis for the risk of SCE and CE in cows examined at 20 days to 30 days postpartum including calving assistance, body score condition, parity, *E. coli* infection, and *Staphylococcus* infection

Variables*	Odds ratio	95% CI	P value
Diagnosis CE			
<i>E coli</i>	7.0	1.05-47.2	0.04
<i>Staphylococcus aureus</i>	10.16	1.66-61.91	0.01
Parity	0.69	0.154-3.08	0.62
Calving	10.6	1.48-76.09	0.02
BCS	2.5	0.32-19.4	0.37
Diagnosis SCE			
<i>E coli</i>	8.6	1.89-39.68	0.03
BCS	11.7	1.24-113.32	0.02
Calving assistance	3.0	0.53-16.6	0.21
Parity	1.93	0.56-6.58	0.28

Variables*: CE: clinical endometritis, SCE: Subclinical endometritis, OR: Odds ratio, BCS: body condition score, infection with *E coli* (0=infection: 1=no infection), infection with *Bacillus* (0=infection: 1=no infection), infection with *Staphylococcus* (0=infection: 1=no infection), calving assistance (0=assisted calving: 1=normal calving): BCS : (0= > 3: 1= ≥ 3), parity : (0=primiparous: 1=multiparous)

Table 5: Results of Cox regression for the hazard of non-pregnancy within 180 days in cows examined 20 days to 30 days after calving

Factors*	No pregnancy		P value
	HR	CL 95%	
CE	1.49	0.66-3.34	0.32
SCE	1.24	0.5-2.94	0.61
<i>E coli</i>	1.3	0.63-2.75	0.51
<i>Bacillus</i>	0.51	0.63-2.75	0.44
<i>Staphylococcus</i>	0.9	0.28-2.91	0.86
Calving assistance	0.51	0.26-1.22	0.15
BCS	0.76	0.42-1.37	0.36

*Factors: CE: clinical CE: clinical endometritis, SCE, Subclinical endometritis, HR, hazard ratio, BCS: body condition score, CE (0=CE: 1=no CE diagnosed), SCE (0=SCE: 1=no SCE diagnosed), infection with *E coli* (0=infection: 1=no infection), infection with *Bacillus* (0=infection: 1=no infection), infection with *Staphylococcus* (0=infection: 1=no infection), calving assistance (0=assisted calving: 1=normal calving), BCS: (0= > 3: 1= ≥ 3)

of these cows (Diskin and Kenny, 2016). The effects of these factors on CE and SCE rates and reproductive

performance were also investigated. The overall occurrence of CE was 11%, which is lower than the range reported by previous studies in cow farms in other countries. Most studies recorded a CE prevalence between 20% to 40% in postpartum cows (Heuwieser et al., 2000; McDougall et al., 2011). The discrepancy in the results may be due to decreased post-calving problems, such as dystocia, retained placenta, and metabolic disorders. Furthermore, most cows in this study depended on grazing and had low milk production, thereby developing few stress factors and minimal exposure to uterine infection after calving. The SCE prevalence in the present study was 12.3%, which is lower than that in a previous study in beef cows (Santos et al., 2009) and other studies in dairy cows (Cheong et al., 2011; Madoz et al., 2013). In one of the previous study in beef cows, 17% of Angus cows (2-78 d postpartum) were positive to endometritis by using low-volume uterine lavage method (Santos et al., 2009).

Thus far, no consensus has been established with regard to the effect of threshold value and time of uterine sampling on SCE diagnosis. SCE can be diagnosed using different cut-off values, such as PMN % range of 5%–18, and various techniques using cytobrush and low-volume lavage (Barlund et al., 2008). Other studies depended on the thresholds of PMN % according to the effects on the reproductive performance (Cheong et al., 2011; McDougall et al., 2011). Kasmanickam et al. (2004) depended on >18% PMNs as threshold value between 20 -33 days postpartum and >10% PMNs between 34 and 47 days postpartum using the cytobrush to diagnosis endometritis while Gilbert et al. (2005) used >5% PMNs as a significant cut-off point for diagnosis endometritis in cows using lavage method between 40 and 60 days postpartum. The low SCE prevalence may be attributed to differences in geographic area, environment, and endometrial cells counted among the studies. A total of 300 cells were counted per slide in the present study, whereas 100 cells were counted in previous studies (Barlund et al., 2008).

E. coli was the common bacteria isolated from healthy (14.6%) and endometritic cows (38.8% in SCE cows and 42.8% in CE cows) in the period between 20 days to 30 days postpartum; these results are either similar to other studies in the early postpartum interval at cow farms (Santos et al., 2011; Sens and Heuwieser, 2013) or higher than those reported in previous studies (McDougall et al., 2011; Prunner et al., 2014a). Most studies confirmed that *E. coli* was isolated in the early period of 0 day to 15 days after calving, and the percentage gradually decreased with the advancement of the postpartum period (Werner et al., 2012). The present results agree with previous study demonstrating that *E. coli* and *A. pyogenes* were the common uterine pathogens in the postpartum period (Sens and

Table 6: Reproductive performance of early postpartum beef cows subsequent to study of vaginal secretions, endometrial cytology, and bacterial isolations

Factor	Total (%)	healthy cows	SCE*	CE**
Number of cows	82	64	9	9
No. of pregnant cows/total, pregnancy % at 150 days post calving	29/82 (35.3)	24/64 (37.5)	3/9 (33.3)	2/9 (22.2)
No. of pregnant cows/total, pregnancy % at 200 days post calving	37/82 (45.1)	30/64 (46.8)	4/9 (44.4)	3/9 (33.3)

* SCE: Subclinical endometritis, **CE: Clinical endometritis

Heuwieser, 2013). Werner et al. (2012) reported the lack of association between the abnormal vaginal discharge and *E. coli* infection in endometritic cows. Although *E. coli* is a common bacteria in the environment, specific strains of this species have been isolated from cows with uterine diseases (Sheldon et al., 2006). Endometrial pathogenic *E. coli* is more adherent and invasive in the endometrium compared with *E. coli* isolated from the uterus of clinically unaffected cows. These pathogenic *E. coli* strains develop diseases of endometrial surfaces such as postpartum metritis or endometritis in the bovine genital tract (Sheldon et al., 2006). In contrast to most studies, the present study showed a decreased number of *A. pyogenes* (one isolate) in CE cases, leading to few cases of purulent vaginal discharge (scores 2 and 3) (Zobel, 2013). However, Sens and Heuwieser (2013) reported in the previous study about bacterial isolation during postpartum period that *E. Coli* and *A. pyogenes* were the dominant bacteria that isolated from uterus between 7-24 days postpartum.

Moreover, *Streptococcus* isolation from cows in the present study was less frequent and did not severely affect CE and SCE; similarly, previous studies reported *Streptococcus* as an opportunistic bacteria in the postpartum uterus (Williams et al., 2005). In contrast to the present findings, Werner et al. (2012) reported that infection with *Streptococcus* during the early postpartum period increased the risk for the occurrence of abnormal vaginal discharge and elevated uterine PMN %. In the present study, *S. aureus* was isolated from CE cows and affected the risk factor for its occurrence. These results are consistent with previous studies, thereby confirming the serious effect of *Staphylococcus* spp. on uterine infection (Prunner et al., 2014b). However, the results were in contrast to other studies reporting the absence of significant effect of *Staphylococcus* on CE cows (Williams et al., 2005).

This study showed the effect of calving assistance (dystocia) as a risk factor on CE and SCE; the results are in agreement with previous studies reporting the effect of calving assistance on the rate of possible uterine contamination after calving (Madoz et al., 2013). Rogers et al. (2004) mentioned in one study that cows which had dystocia has more chances to suffer from uterine infections and increased culling rate in the future than those

that calved without assistance. However, this result is in contrast with another study that did not find any effect of calving assistance on CE and SCE occurrence (Prunner et al., 2014a).

In the present study, no association was found between BCS and CE occurrence; similarly, previous studies that failed to demonstrate any effect of BCS on CE (Potter et al., 2010). However, Cheong et al. (2011) reported that BSC significantly affected the rate of CE in cows. The present study also demonstrated the effect of BCS on SCE occurrence, which may be attributed to the effect of negative energy balance (NEB) after calving on the immunity of cows, thereby increasing the probability of uterine infection (Gilbert et al., 2005). Hammon et al. (2006) mentioned that uterine infection was accompanied with NEB, which begins before birth and continues through the early lactation; this study also reported that cows with acute NEB exhibit reduced neutrophil function and developed SCE. Body energy reserves at calving consider an essential factor influencing reproductive performance in beef cattle, and it is a most important factor determining when beef heifers and cows will resume cycling after calving and response to postpartum nutrient intake (Wettemann et al., 2003).

In the present study, poor to moderate agreement was found among PMN %, bacteriological findings, and vaginal discharges; previous results indicated that abnormal discharges do not necessarily indicate uterine infection (Dubuc et al., 2010; Westermann et al., 2010). The present study also showed poor agreement between bacterial findings and PMN, similar to the findings of other studies (Barański et al., 2012). Not all of the bacterial species cause inflammation and infusion of PMN to the uterine endothelium because numerous bacteria may be normal inhabitants of the uterus; uterine infection after calving can also be due to other causes, such as yeast and viruses. Reproductive performance is one of the most common economically important traits in beef production (Bormann et al., 2006).

The current study showed minimal effects of CE, SCE, and isolated bacteria on the reproductive performance of cows because the ability of these cows to self-cured; this finding

is similar to those reported in a previous study (Prunner et al., 2014b). However, most studies showed increased days open and days to conception in cows suffering from uterine diseases, such as CE and SCE (Elkjær et al., 2013; Madoz et al., 2013). The decreased number of acute infection cases (scores 3) of the endometrium caused by *A. pyogenes* could result in the reduction of acute destruction of the endometrium layer and impairment of future reproductive performance of these cows. Most of the beef cow herds exhibit high pregnancy rate during the breeding season that they are properly managed (Amundson et al., 2006) and uterine contamination can be controlled especially after the resumption of the ovarian cycle (Santos et al., 2009). The current study also showed a decline in the pregnancy rate of beef cows 45.1 % (37/82) at 200 days post calving because several possible factors affect the reproductive performance of these cows, such as seasonal breeding, prolonged postpartum acyclicity in suckled beef cows, nutrition, breed and uterine infection. Pregnancy rates in the day 200 after birth was more than at 150 days, the reason for that may be these cows have a longer period to overcome their reproductive problems and more chance to be pregnant. Extended post calving anestrus in suckled beef cows is one of the most common limitations to gaining a calf every year (Miller and Ungerfeld, 2008). Climatic stress, parity, extended suckling, nutritional deficiencies, and management practices were the most common reasons of prolonged calving intervals (Diskin and Kenny, 2016).

Further studies are needed to shed light on the uterine infections in beef cows and its relationship to other factors that cause weakness of the reproductive performance of these cows.

CONCLUSION

A moderate agreement exists among PMN %, bacteriological findings, and vaginal discharges, whereas a poor agreement exists between bacterial findings and PMN %. *E. coli*, *S. aureus*, and calving assistance (dystocia) were the major factors affecting uterine infection in beef cows. CE and SCE insignificantly affected the reproductive performance of beef cows.

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Author Contributions

All authors contributed effectively in this research, *N. Salah* designed and achieved experiments and wrote the paper; *B. Siti kbairani* performed bacteriological part of the work; *M.A.Omar* analysed the data; *N. Yimer*, *H. Wahid*, and *Y. Rosnina* supervised the project and revised the manuscript.

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