Research Article

Treatment of Endometritis Caused by Different Types of Bacteria by Cefoperazone Antibiotic and its Effect on the Days Open and Pregnancy Rate in Dairy Cows (Field Study)

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Abstract | One of the most important problems on dairy farms is postpartum endometritis. It can destroy the future of dairy farms and the dairy industry since it leads to prolonged days open and prolonged intercalving intervals, resulting in involuntary culling. The current study aimed to detect the most sensitive antibiotic to different types of microorganisms responsible for endometritis and its effect on the days open and pregnancy rate. The current study was carried out on 100 Holstein Frisian cows that were not pregnant for 90-120 days after calving. The cows were classified into two groups: the first group is the normal control group (9 cows), and the second group (91 cows) is the treated group and suffered from endometritis. Samples for bacteriological examination were taken and subjected to antibiotic sensitivity tests. The most sensitive antibiotic was used for the treatment of the infected group. After the end of the treatment protocol, the herd reproduction management was applied to all treated cows. Results showed that different bacterial species were isolated (*Staphylococcus, Streptococcus, Escherichia coli,* and *Klebsiella*). The infection is either a single infection (*Staphylococcus* only), or a mixed infection, while the control group has no bacterial growth. *Staphylococcus* was the only microorganism responsible for the single infection. Infection with *Staphylococcus* and *E. coli* has a higher incidence of infections with two microorganisms, while infection with *Streptococcus* and *Klebsiella* has the least incidence of infections with two microorganisms. The results also revealed that Cefoperazone is the most sensitive antibiotic. Cows infected with mixed infections have the longest postpartum period of pregnancy, especially those infected with *E. coli*, and *Klebsiella*. It is concluded that the greater the number and virulence of the microorganisms responsible for the infection, the greater the severity of uterine inflammation and the longest postpartum period of pregnancy. Various types of bacteria are responsible for endometritis, such as *Staphylococcus, Streptococcus, E. coli, and Klebsiella*. Cefoperazone is the most sensitive antibiotic for the treatment of complicated cases of postpartum endometritis caused by mixed infections and high virulence bacteria.

Keywords | Endometritis, Bacterial infection, Cefoperazone treatment, Dairy cows, Days open, Pregnancy rate.

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INTRODUCTION

Inflammation of the endometrium of dairy cows after parturition by 21 days or more without clinical signs is known as postpartum endometritis (Sheldon et al., 2006; Kusaka et al., 2020). Endometritis has been shown to reduce first-service conception rate and overall pregnancy risk (Barlund et al., 2008, Galvão et al., 2009, Cheong et al., 2011). Now, up to 40% of dairy cattle develop postpartum uterine disease every year, ranging from subclinical endometritis to life-threatening metritis (Sheldon et al., 2019). Recent research concerned with endometritis indicates that the economic impact of endometritis may reach millions of pounds annually (McNaughton, & Murray, 2009). Endometritis can be detected earlier by investigating the presence of purulent vaginal exudate, which poses the same deleterious effect on fertility (Runciman et al., 2008, Runciman et al., 2009, Dubuc et al., 2011). It is also evidenced that infertility associated with lactation may result from endometritis (Salasel et al., 2010). Endometritis does not only reduce fertility by the direct consequence of the inflammation in the uterus and oviduct but is also caused by the abnormal oestrous cycles and damaged oocytes occurring as a result of endometritis (Bromfield et al., 2015).

It was reported that the first fourteen days postpartum (PP) of dairy cattle are associated with a high bacterial contamination rate, reaching 80 - 90% of the cows (Sheldon et al., 2009). Failure to avoid contamination harms uterine function, causing clinical endometritis, which results from the persistence of pathogenic bacteria for at least 3 weeks postpartum (LeBlanc et al., 2002, Sheldon et al., 2009). Most outbreaks of clinical endometritis involve tissue damage, delayed uterine involution, disruption of endometrial function, and perturbation of ovarian cycles (Opsomer et al., 2000, Sheldon et al., 2009). Therefore, avoiding the incidence of endometritis requires a normal postpartum period, which is characterized by the prompt involution of the uterus and regeneration of damaged endometrium, the resumption of ovarian cyclical activity, and the control of pathogenic bacteria in the uterus (Sheldon et al., 2019). Future work should focus on determining which bacteria and virulence factors cause endometritis, and understanding how the host response to infection is regulated in the endometrium to develop strategies to limit the impact of uterine disease on fertility (Carneiro et al., 2016). Moreover, treatment of endometritis is urgent to overcome all pathogenic bacteria without causing significant hazard effects. Furthermore, treatment should be safer, not affect reproductive performance, and significantly decrease the time consumed to achieve pregnancy. Therefore, this study concerns with the determination of bacteria causes detrimental effects of inflammation of the female bovine reproductive tract on the establishment and maintenance of pregnancy. In addition to detecting the most sensitive antibiotic to different types of bacteria responsible for endometritis and its effect on the days open and pregnancy rate.

MATERIALS AND METHODS

Animal Housing

One hundred Holstein Friesian dairy cows (primipara) ranging from 2.5-3 years with a body condition score of more than 3.5 were used in the present study (scale: 1 = thin, 5 = fat) (Bhalaru et al., 1987). The cows are located on a governmental dairy farm in the south of Egypt (Qena province). All cows were dried well 75 days before calving. The cows were kept in an open shed with a concrete floor. The main feeding stuff during the winter was barssem, rice straw, and concentrate mixture, while during the summer season, drawa was used instead of barseem. All the cows were bred by natural serving.

All experimental procedures were approved by the Ethics Committee for Animal Experiments of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Clinical Examination of the cows

Clinical endometritis (CE) is defined as an inflammation of the endometrium occurring later than 21 days in milk (DIM) with purulent vaginal discharge and a cervical diameter of >7.5 cm or mucopurulent discharge after 26 DIM (LeBlanc et al., 2002a; Leutert et al., 2012).

Each cow was examined at the time of a routine gynaecological examination after calving. Non pregnant cows after 90 -120 days from the last calving were gynecologically examined, including rectal palpation and vaginal examination. The vaginal examination was performed and the nature of the vaginal discharge was detected. With the help of a flashlight, the cervix and vagina were visually examined for the presence and quality of discharge as previously described (Leutert et al., 2012). A 4-point scoring system (1 = clear mucus, 2 = mucus containing flecks of pus, 3 = discharge containing less than 50% pus, 4 = discharge containing more than 50% pus) to classify vaginal mucus was established by Williams et al. (2005) and has been used in recent studies (Sheldon et al., 2006; Dubuc et al., 2010; Kaufmann et al., 2010; Šavc et al., 2016). Grade 1 (clear mucus) was considered a normal vaginal secretion while grades 2, 3 and 4 were considered abnormal vaginal secretions. The intervals from parturition to the onset of ovarian activity, to the first observed estrous, and to conception were recorded for each cow.

Sampling for bacteriological isolation

Samples were collected for bacteriological examination immediately after clinical examination. Cows were classified into two groups. The first group is the control group (9 cows), while the second group is the endometritis group or treated group (91 cows). The endometritis group has a history of abnormal calving (dystocia) followed by a retained placenta and failure to conceive after natural serving within 90-120 days of calving. For each cow, the vulva lips were parted and swabs were collected from the uterine body. A transport medium composed of thioglycollate broth was used for transportation of the swabs at 4°C in an icebox and processed for bacteriological examination. It was cultured within 1 h of collection. Samples cultured on Nutrient broth, Nutrient media, Macconkey media, Blood agar, Mannitol media and EMB agar (Lab M Ltd., Lancashire, United Kingdom). Bacteria were identified after a 24-h incubation at 37°C for bacterial growth. Identification was based on the characteristics of colonies, hemolysis, gram stain, morphology, the catalyze, indole production, methyl red, Voges-Proskauer, and citrate production tests (Williams et al., 2005). All isolates were tested for in vitro antibiotic sensitivity (Muneer et al., 1991). Sensitivity to antibiotics was tested by the disc diffusion method and performed according to CLSI (formerly NCCLS) guidelines in Mueller-Hinton agar. For this purpose, a separate disc containing Cefoprazone 10 mg, Lincomycin 10 mg, Tetracycline 10 mg, Ampicillin/sulbactam 10 mg, Tobramycin 10 mg, and Pefloxacin 10 mg per disc was employed.

Methods of treatment

Cows with clinical endometritis were subjected to the treatment regimen using the most sensitive antibiotic to the causative microorganisms (Cefoprazone 4 gm) as the intrauterine route and Cefoprazone 4 gm intramuscular every 24 hours for 5 days.

Detection of uterine involution

The determination of uterine involution is based on gynaecological examinations at three times with a 14-day interval (days 14, 28, and 42) according to the following procedures: evaluation of the general health, detection of the nature of the vaginal secretion, palpation of the uterus, cervix, and ovaries. The uterus is considered completely involuted if the two horns of the uterus are symmetrical and all the uterine parts are present at the edge of the pelvic cavity with normal tonus, and consistency, in addition to the absence of pathological cervical discharge. Based on the case definition of endometritis derived previously, clinical resolution was subsequently defined as the absence of mucopurulent or worse discharge and cervical diameter of 2 fingers at the second examination.

Follow-up Examination was done 14 days after treatment. Clinical cure was detected by the absence of pus discharge. A second dose of treatment can be given to cases that are not clinically cured, and a further examination of these cases occurs for a third and final time after an additional 14 days. The presence of any turbid discharges at the third examination makes the case considered a clinical failure. Normal herd reproduction management was done on all cows after the curing of endometritis. This was achieved by the administration of one or more injections of PGF2α and or GnRH for estrus detection, synchronisation, or synchronisation of ovulation. The following data was recorded to evaluate the reproductive performance, which is: the interval from calving to the first estrus, the interval from calving to pregnancy (days open), and the number of services per conception. Transrectal palpation at 45 days post-breeding was done for pregnancy diagnosis.

Statistical analysis

The collected data was statistically analyzed. Statistical evaluation of the results was performed using SPSS® (SPSS 20, IL, USA). The results for the reproductive findings of the groups and the types of bacterial infections were analyzed using multivariate analysis (ANOVA). A P value < 0.05 was considered statistically significant.

RESULTS

Incidence of endometritis and isolation of different bacteria

The average incidence of endometritis was 91%, depending on the bacterial growth. It is shown in Table (1) that various species of bacteria were isolated from uterine samples, which include *Staphylococcus*, *Streptococcus, E. coli* and *Klebsiella*. The infection is either a single infection (*Staphylococcus* only) (7 cows) or mixed infection (84 cows) while the control group has no bacterial growth (9 cows). It was found that the incidence of the mixed infection is higher than that of the single infection. *Staphylococcus* was the only microorganism that caused a single infection, while the other microorganisms were usually mixed. Mixed infection was classified into three types: infection with two microorganisms, infection with three microorganisms, and infection with four microorganisms. Mixed infection with two microorganisms has a higher incidence. The infection with *Staphylococcus* and *E. coli* is the higher incidence for the infection with two microorganisms (30 cows) (33%), while the infection with *Streptococcus* and *Klebsiella* is the least in incidence in infection with two microorganisms (only one cow). In the infection with three microorganisms, infections with *Staphylococcus, E. coli* and *Klebsiella* had the higher incidence (10 cows), while infections with *Staphylococcus, Streptococcus* and *Klebsiella* had the lower incidence (only one cow).

The effect of endometritis on uterine involution

The comparison between the groups of bacterial infections indicates that the *Staphylococcus* microorganism alone does not cause turbidity in the secretion, while in combination with other microorganisms like *Streptococcus, E.coli,* and *Klebsiella*, the turbidity was obvious. The turbidity varies according to the type of microorganisms, as it is low in the case of infection with *Staphylococcus* in combination with other microorganisms and moderate in the infection with *Streptococcus* in combination with *E.coli*, and reaches its maximum range in the infection of *E.coli* in combination with *klebsiella,* or the infection with three microorganisms or four microorganisms (Table 2).

The vaginal mucosa does not have congestion or has a light degree of congestion in the control group. While congestion was obvious in the case of infection with Staphylococcus microorganism alone (28.7%).The congestion increases in the case of infection with two microorganisms, such as infection with (*Streptococcus* and *E.coli*) (77 %), which was more prominant than infection with (*Staphylococcus* and *Streptococcus*), (*Staphylococcus* and *E.coli*) or (*Staphylococcus* and *Klebsiella*) (50%). It reaches its maximum (100 %) in the case of infection with (*E.coli* and *Klebsiella*) or infection with three microorganisms (*Staphylococcus*, *Streptococcus* and *E.coli*), (*Staphylococcus, E.coli* and *Klebsiella*) or four microorganisms (*Staphylococcus, Streptococcus, E.coli* and *Klebsiella*) (Table 2).

The cervix was slightly inflamed (slightly thick) in the case of infection with the Staphylococcus microorganism (28.7%) while this inflammation increased when the infection becomes combined with two microorganisms (50%) such as (*Staphylococcus* and *Streptococcus*), (*Staphylococcus* and *Klebsiella*). The inflammation also increases more in infection with (*Staphylococcus* conjugated with *E.coli*) (85%) or (*Streptococcus* conjugated with *E.coli*) to reach its maximum (100%) in infection with (*E.coli* conjugated with *klebsiella*) or infection with three microorganisms (*Staphylococcus, Streptococcus* and *E.coli*), (*Staphylococcus, E.coli* and *Klebsiella*) or four microorganisms (*Staphylococcus, Streptococcus*, *E.coli* and *Klebsiella*) (Table 2).

The uterine horn was slightly inflamed (slightly thick and asymmetrical) in the infection with *Staphylococcus* microorganism (single infection), where this inflammation increased in the infection with two microorganisms depending on the virulence of the combined microorganisms, as it is higher in the case of infection with (*Staphylococcus, E. coli*) or (*Streptococcus, E. coli*) (77%) than the infection with (*Staphylococcus, Streptococcus*) or (*Staphylococcus, Klebsiella*) (50 %). The inflammation reaches its maximum in the case of infection with (*E. coli, Klebsiella*) or infection with three microorganisms (*Staphylococcus, Streptococcus* and *E.coli*), (*Staphylococcus, E.coli* and *Klebsiella*) or four microorganisms (*Staphylococcus, Streptococcus*, *E.coli* and *Klebsiella*) (Table 2).

Ovarian structures in relation to the type of bacterial infection

The clinical findings in the ovary in relation to the type of bacterial infection were shown in Table 2. The corpus luteum was the only structure found in the ovary in the cases of single infection with *Staphylococcus* microorganism, mixed infection as (*Staphylococcus*, *Streptococcus*) and/or (*Staphylococcus, Streptococcus, E. coli* and *Klebsiella*) (100%). The presence of the corpus luteum was higher in cases of infection with (*Streptococcus, E. coli*) where its incidence was (85%) and the rest (15%) was cystic ovaries. The incidence of CL in the aforementioned group was higher than its incidence in cases of infection with (*Staphylococcus*, *E. coli*) (70% CL and 30% cystic ovary), and higher than the incidence of infection with (*E. coli*, *Klebsiella*), (*Staphylococcus, Streptococcus, E. coli*) (75% CL and 25% cystic ovary). The presence of cystic ovary was prominent in cases of infection with (*Staphylococcus, Klebsiella*) or (*Staphylococcus, E. coli, Klebsiella*) as it occurs in 50 % of the infected cases while the other 50% have CL (Table 2).

The postpartum period in relation to the type of bacterial infection

The number of the cows treated in each dose was indicated in Table 3. The postpartum period to pregnancy (days open) was short in cases of single infection with *Staphylococcus* microorganism and mixed infection with *Streptococcus* and *Klebsiella,* but moderate in cases of mixed infection with two microorganisms such as (*Staphylococcus*, *Streptococcus*) and (*Staphylococcus, Klebsiella*). The postpartum period becomes longer in mixed infection with (*Staphylococcus, E.coli*) and (*Streptococcus, E.coli*). This period increases more in mixed infections with (*E.coli, Klebsiella*) and in mixed infections with three microorganisms like (*Staphylococcus, Streptococcus,* and *E .coli*) and (*Staphylococcus, E.coli,* and *Klebsiella*) to reach its maximum in mixed infection with four microorganisms (*Staphylococcus, Streptococcus, E.coli,* and *Klebsiella*) (Table 4).

The pregnancy rate was higher in cases of infection such as (*Staphylococcus* infection), mixed infections such as (*Staphylococcus, Streptococcus*) and (*Staphylococcus, Streptococcus, E. coli* and *Klebsiella*) (100%), while this rate decreased to moderate in cases of mixed infections such as (*Staphylococcus, Klebsiella*) or (*Staphylococcus, E. coli* and *Klebsiella*) (50%). The pregnancy rate was at a level above moderate in cases of mixed infection by (*Streptococcus, E .coli*) (85%), (*E. coli, Klebsiella*) (75%), (*Staphylococcus, Streptococcus, and E. coli*) (75%), and (*Staphylococcus, E. coli*) (70%) (Table 4).

Table 1: Bacteriological findings of the uterine swab and the most sensitive antibiotic

Type of infection	No. of bacterial infection	Name of microorganisms	No. of +ve samples by culture	The most sensitive antbiotic
Single	with one microorganism	*Staphylococcus* only	7	Cefoperazone , ampicillin/sulbactam
Mixed	with two microorganisms	*Staphylococcus* and *Streptococcus*	2	Cefoperazone , ampicillin/ sulbactam
		Staphylococcus and *E.coli*	30	Cefoperazone, pefloxacin
		Streptococcus and *E.coli*	13	Cefoperazone, pefloxacin
		Staphylococcus and *Klebsiella*	2	Cefoperazone, pefloxacin
		Streptococcus and *Klebsiella*	1	Cefoperazone, pefloxacin
		E.coli and Klebsiella	5	Cefoperazone, pefloxacin
	with three microorganisms	*Staphylococcus, Streptococcus* and *E.coli*	8	Cefoperazone, pefloxacin
		Staphylococcus, Klebsiella and *E.coli*	10	Cefoperazone, pefloxacin
		Streptococcus, Klebsiella and *E.coli*	2	Cefoperazone, pefloxacin
		Staphylococcus, Streptococcus and *Klebsiella*	1	Cefoperazone, pefloxacin
	with four microorganisms	*Staphylococcus, Streptococcus, E.coli* and *Klebsiella*	10	Cefoperazone, pefloxacin
CONTROL	No bacterial growth		9	
TOTAL			100	

Table 2: The reproductive clinical findings in relation to the type of bacterial infection of dairy cows:

Type of bacterial infection	Vaginal secretion		Vaginal Mucosa		Cervix diameter		Uterine horn diameter		Ovarian Findings	
	Normal discharge	Abnormal discharge	Rosy red	Congested	Two fingers	Three fingers	Inflamed (3 fingers)	Non inflamed (2 fingers)	CL	Cyst
Control	100 [a]	0	75 [a]	25 [d]	75 [a]	25 [d]	25 [d]	75 [a]	100 [a]	0
Staphylococcus only	100 [a]	0	71.3 [a]	28.7 [d]	71.3 [a]	28.7 [d]	28.7 [d]	71.3 [a]	100 [a]	0
Staphylococcus and *Streptococcus*	50 [b]	50 [c]	50 [b]	50 [c]	50 [b]	50 [c]	50 [c]	50 [b]	90 [b]	0
Staphylococcus and *E. coli*	50 [b]	50 [c]	50 [b]	50 [c]	15 [c]	85 [b]	77 [b]	23 [c]	70 [c]	30 [b]
Staphylococcus and *Klebsiella*	50 [b]	50 [c]	50 [b]	50 [c]	50 [b]	50 [c]	50 [c]	50 [b]	50 [d]	50 [a]
Streptococcus and *E. coli*	23 [c]	77 [b]	23 [c]	77 [b]	15 [c]	85 [b]	77 [b]	23 [c]	85 [c]	15 [c]
E. coli and *Klebsiella*	0	100 [a]	0	100 [a]	0	100 [a]	100 [a]	0	75 [c]	25 [c]
Staphylococcus, Strepto-coccus and *E. coli*	0	100 [a]	0	100 [a]	0	100 [a]	100 [a]	0	75 [c]	25 [c]
Staphylococcus, E. coli and *Klebsiella*	0	100 [a]	0	100 [a]	0	100 [a]	100 [a]	0	50 [d]	50 [a]
Staphylococcus, Streptococcus, E. coli and *Klebsiella*	0	100 [a]	0	100 [a]	0	100 [a]	100 [a]	0	100 [a]	0

- The results are expressed as a percentage.
- CL: Corpus luteum
- Values bearing different superscripts in the same column differ significantly ($P<0.05$)

Table 3: The number of the cows treated in each dose

Total No. of cows	No. of cows for 1st dose	No. of cows for 2nd dose	No. of cows for 3rd dose	Resist
91	21/91	20/70	30/50	20

Table 4: Fertility parameters (pregnancy rate and days open) of dairy cows in relation to the type of bacterial infections:

Type of bacterial infection	Total number of the cases	Pregnancy rate after treatment		Mean Postpartum days to pregnancy
		Pregnant	Non Pregnant	
Control	9	100	0	113
Staphylococcus only	7	100	0	145
Staphylococcus and *Streptococcus*	2	100	0	150
Staphylococcus and *E. coli*	30	70	30	170
Staphylococcus and *Klebsiella*	2	50	50	160
Streptococcus and *Klebsiella*	1	100	0	145
Streptococcus and *E. coli*	13	85	15	180
E. coli and *Klebsiella*	5	75	25	245
Staphylococcus, Streptococcus and *E. coli*	8	75	25	245
*Staphylococcus, Streptococcus,*and *Klebsiella*	1	0	100	245
Staphylococcus, E. coli and *Klebsiella*	10	50	50	250
Streptococcus, *Klebsiella* and *E. coli*	2	50	50	250
Staphylococcus, Streptococcus, E. coli and *Klebsiella*	10	100	0	275

-The values of pregnancy rate after treatment are expressed as a percentage.

DISCUSSION

Shortly after parturition, the uterus is a large organ and the normal involution is an aseptic process, but this is the exception rather than the rule, as the uterus that suffers from periparturient problems is larger and cannot control uterine infections. If dystocia occurs or if assistance is needed, the chances of bacterial contamination increase. Dystocia or uncorrected obstetrical manipulations cause excessive stretching of the uterus, which reduces its contractility. Consequently, retention of lochia occurs beyond the normal period, which exposes a good medium for bacterial growth and eventually causes endometritis.

The present work showed that the average incidence of endometritis was 91%. This result agrees with several previous studies that indicated mild to severe endometritis occurred in 90% of postpartum cows during the second to fourth postpartum weeks (Földi et al., 2006, Sheldon et al., 2004).

The results of the present study showed a wide variety of bacteria isolated from cows infected with PP endometritis. The bacteria most frequently isolated are *Escherichia coli* (*E. coli*), *Staphylococcus spp*, *Streptococcus spp*, and *Klebsiella spp*. The most common pathogenic species are *E. coli* and *Staphylococcus* spp. and this infection was mainly due to retained foetal membrane. Other bacteria, such as *Streptococcus spp*. have also been isolated as additional flora in connection with major uterine pathogens. These results are somewhat consistent with previous studies (Sheldon et al., 2004, Williams et al., 2005). These studies illustrated that the bacteria isolated were found to be obligate uterine pathogens (*E. coli, Trueperella pyogenes*), potential uterine pathogens (non-hemolytic *streptococci*) and opportunistic bacteria (*Klebsiella spp., Proteus spp.* and *Staphylococci*). Königsson et al. (2001) reported that *E.coli, Streptococci, Fusobacterium necrophorum (F. necrophorum),* and *Trueperella pyogenes*, were predominant bacteria in cows with endometritis resulting from retained foetal membranes. Later on, (Königsson et al., 2001, Williams et al., 2005, LeBlanc, 2008) demonstrated that *E. coli* and *Streptococcus spp*. are the most commonly isolated bacterial agents from the uterus of postpartum cows. However, the same authors reported that *E. coli, A. pyogenes, F. necrophorum* and *Prevotella* species were the most common and major uterine pathogens. Other bacteria, such as *Streptococci, Staphylococci,* and *E. coli,* have also been cultured and identified as having endometritis of varying severity (Zemjanis, 1980). It has been difficult to identify the most significant microbes associated with endometritis because both pathogenic and non-pathogenic organisms colonise the bovine endometrium, and many of them are fastidious (Studer, 1981).

It is noticed in the present work that some bacteria act synergistically to enhance the severity of uterine diseases like those occur in mixed infections with *E. coli* and *Staphylococcus*, and that of *Streptococcus* and *E. coli*. These results are close to the results stated by Singh et al. (2008) in which some bacteria, including *A. pyogenes, F. necrophorum*, and *Prevotella spp*., act synergistically to enhance the severity of the uterine disease. Each of these species produces substances to enhance bacterial growth. Some bacteria, such as *F. Necrophorum,* after invading the uterine tissue, produce a leucocidal toxin that suppresses phagocytosis (Singh et al., 2008, Sheldon, 2004) and protects *A. Pyogenes*, which subsequently produces catalase and a growth factor to support the proliferation of *F. Necrophorum* (Singh et al., 2008).

Under routine field conditions, it is suggested that herd records are first used to identify cows at risk of endometritis, such as cows with a history of retained placenta, dystocia, twins, hypocalcaemia, ketosis, or metritis. Then, the vaginal mucus should be examined for the presence of pus (Sheldon, 2020). The types of bacteria responsible for endometritis reflect the appearance and degree of turbidity of the uterine secretion. The present work found infection with *Staphylococcus* only has a clear secretion while combinations between *Staphylococcus* and *E. coli*, or *Streptococcus,* or *Klebsiella* cause turbidity of the secretion. Mixed infections usually cause turbidity of the secretion, which varies according to the severity and type of mixed microorganisms. Mixed infection with three microorganisms causes more turbidity than infection with two microorganisms, while infection with four microorganisms has the highest degree of turbidity. In the previous study, Williams et al. (2005) evaluated the appearance and odour of vaginal mucus and showed that mixed infections associated with purulent or mucopurulent discharge, while other mixed infections associated with a change in the odour of exudate, such as *E. coli,* non-hemolytic *Streptococci,* and *A. pyogenes*, cause foul-smelling exudates. Furthermore, several previous studies have also illustrated that clinical endometritis is characterised by the presence of purulent discharge excreted from the uterus. (Sheldon et al., 2002, Runciman et al., 2009, Pleticha et al., 2009). Recently, an experimental study was carried out on an in vivo model of clinical endometritis in Holstein heifers using pathogenic *E. coli* and *Trueperella pyogenes* (*T. pyogenes).* The study showed that heifers that received intrauterine infusion of pathogenic bacteria developed purulent vaginal mucus, an accumulation of echogenic fluid in the uterus, and an increased bacterial load in vaginal mucus (Piersanti et al., 2019).

Uterine involution necessitates a reduction in cervical diameter from approximately 30 cm immediately following parturition to approximately 2 cm at day 7 postpartum. Kasimanickam et al. (2004) found that the larger the diameter of the cervix after parturition, the longer the time taken for involution to occur. The results of our study indicate that cows suffering from endometritis have abnormalities in their cervix and uterine horn (very thick and more than 7.5 cm) while normal cows (control group) have a normal cervix (less than 7.5 cm). The results of the present study are in line with those of the previous one (Oltenacu et al., 1983) which mentioned that the diameter of the cervix was greater than 5.5 cm in primiparous and 6.0 cm in multiparous cows detected by rectal palpation 12 to 26 days postpartum was the best indicator of subsequent poor reproductive performance.

In the present work, it was found that cows bearing CL on the ovary have a decreased days-open and an increased pregnancy rate relative to cows with a cyst on the ovary or no palpable structures. These results are somewhat in accordance with that of Bonnett et al. (1993), who illustrated that cows that posed an early resumption of the ovary during the postpartum period at 26 days in milk acquired a 10 % increase in the pregnancy rate. Uterine infection postpartum could raise the secretion of PGF2α and cortisol, which is accompanied by the development of cystic ovarian disease (Etherington et al., 1985a, Etherington et al., 1985b, Bosu and Peter, 1987). This is also confirmed by the results of the current study, as some cows with uterine infection (endometritis) have cysts on their ovaries (21% of the cows) while the other cows have a CL.

In this study, the postpartum period to pregnancy (days-open) was increased due to endometritis and reached more than 125 days in cows infected with two microorganisms (mild endometritis) and reached 245 days in cows infected with four microorganisms (severe endometritis). The pregnancy rate reached approximately 78 %, in contrast, 22% of the cows were empty and they will be culled due to reproductive failure. These findings are in the same line with previous studies. McDougall et al. (2011) and Lee and Kim (2006) showed reduced pregnancy rates and subsequently increased culling rates in cows with uterine infection, while Gilbert et al. (2005) found endometritis was highly significant for reduced pregnancy rates where days open increased to 218 days.

CONCLUSION

It is concluded that the greater the number and virulence of the microorganisms responsible for the infection, the greater the severity of uterine inflammation and the longest PP period to pregnancy. Various types of bacteria are responsible for uterine infections causing endometritis, such as *Staphylococcus*, *Streptococcus, E. coli,* and *Klebsiella*. Cefoperazone is the most sensitive antibiotic for the treatment of complicated cases of postpartum endometritis caused by mixed infections and high-virulence bacteria. In addition, cefoperazone treatment hurries the uterine involution and, subsequently, decreases the days-open and increases the pregnancy rate.

Further researches should be carried out on cefoperazone treatment to discover its sensitivity against other types of microorganisms responsible for endometritis in animals and humans.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

NOVELTY STATEMENT

The current study showed that different bacterial species (*Staphylococcus, Streptococcus, Escherichia coli,* and *Klebsiella*) were isolated from cows suffering from postpartum endometritis. Cefoperazone is the antibiotic that is sensitive to these types of microorganisms responsible for endometritis and has a favorable effect on the days open and pregnancy rate.

AUTHORS CONTRIBUTION

Yahia, Alaa, Mohamed and Abd El-Latif conceived and designed the study. Yahia executed the experiment and interpreted the data, seriously revised the manuscript for necessary intellectual contents, and approved the final manuscript. Waleed and Yahia revised the manuscript. All the authors have approved the submitted version of the manuscript.

REFERENCES

Barlund C, Carruthers T, Waldner C, Palmer C. (2008). A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology. 69(6):714-723 https://doi.org/10.1016/j.theriogenology.2007.12.005.

Bhalaru, S., Tiwana, M., & Singh, N. (1987). Effect of body condition at calving on subsequent reproductive performance in buffaloes. Indian J. Anim. Sci. (India).(57):33-6.

Bonnett BN, Martin SW, Meek AH. (1993). Associations of clinical findings, bacteriological and histological results of endometrial biopsy with reproductive performance of postpartum dairy cows. Prevent. Vet. Med. 15(2-3):205-220 https://doi.org/10.1016/0167-5877(93)90114-9.

Bosu W, Peter AT. (1987). Evidence for a role of intrauterine infections in the pathogenesis of cystic ovaries in postpartum dairy cows. Theriogenology. 28(5):725-736. https://doi.org/10.1016/0093-691X(87)90289-5

Bromfield J, Santos JP, Block J, Williams R, Sheldon I. (2015). Physiology and endocrinology symposium: Uterine infection: linking infection and innate immunity with infertility in the high-producing dairy cow. J. Anim. Sci. 93(5):2021-2033. https://doi.org/10.2527/jas.2014-8496

Carneiro LC, Cronin JG, Sheldon IM. (2016). Mechanisms linking bacterial infections of the bovine endometrium to disease and infertility. Reproductive biology 16(1):1-7. https://doi.org/10.1016/j.repbio.2015.12.002

Cheong S, Nydam D, Galvão K, Crosier B, Gilbert R. (2011). Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. J. Dairy Sci. 94(2):762-770. https://doi.org/10.3168/jds.2010-3439

Dubuc J, Duffield T, Leslie K, Walton J, LeBlanc S (2011). Randomized clinical trial of antibiotic and prostaglandin treatments for uterine health and reproductive performance in dairy cows. J. Dairy Sci. 94(3):1325-1338. https://doi.org/10.3168/jds.2010-3757

Dubuc J, TF Duffield, KE. Leslie, JS Walton, SJ LeBlanc (2010). Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93:5764–5771. https://doi.org/10.3168/jds.2010-3429

Etherington W, Martin S, Dohoo IR, Bosu W (1985a). Interrelationships between ambient temperature, age at calving, postpartum reproductive events and reproductive performance in dairy cows: a path analysis. Canadian J. Comparat. Med. 49(3):254.

Etherington W, Martin S, Dohoo IR, Bosu W. (1985b). Interrelationships between postpartum events, hormonal therapy, reproductive abnormalities and reproductive performance in dairy cows: a path analysis. Canadian J. Comparat. Med. 49(3):261.

Földi J, Kulcsar M, Pecsi A, Huyghe B, De Sa C, Lohuis J, Cox P, Huszenicza G. (2006). Bacterial complications of postpartum uterine involution in cattle. Anim. Reprod. Sci. 96(3-4):265-281. https://doi.org/10.1016/j.anireprosci.2006.08.006

Galvão K, Frajblat M, Brittin S, Butler W, Guard C, Gilbert R.(2009). Effect of prostaglandin F2α on subclinical endometritis and fertility in dairy cows. J. Dairy Sci. 92(10):4906-4913. https://doi.org/10.3168/jds.2008-1984

Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M (2005). Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 64(9):1879-1888. https://doi.org/10.1016/j.theriogenology.2005.04.022

Kasimanickam R., Duffield T., Foster R., Gartley C., Leslie K., Walton J., Johnson W (2004). Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum Dairy Cows. Theriogenology., 62: 9-23.

Kaufmann TB, S Westermann, M Drillich, J Plontzke, W Heuwieser (2010). Systemic antibiotic treatment of clinical endometritis in dairy cows with ceftiofur or two doses of cloprostenol in a 14-d interval. Anim. Reprod. Sci. 121:55–62. https://doi.org/10.1016/j.anireprosci.2010.04.190

Königsson K, Gustafsson H, Gunnarsson A, Kindahl H (2001). Clinical and Bacteriological Aspects on the Use of Oxytetracycline and Flunixin in Primiparous Cows with Induced Retained Placenta and Post-partal Endometritis. Reprod. Domest. Anim. 36(5):247-256. https://doi.org/10.1046/j.1439-0531.2001.00289.x

Kusaka H, Hasegawa R, Nishimoto N, Kawahata M, Miura H, Kikuchi M, Sakaguchi M. (2020). Comparison of diagnostic methods for uterine health in dairy cattle on different days postpartum. Vet. Rec. 186(3):91-91. https://doi.org/10.1136/vr.105300

LeBlanc S, Duffield T, Leslie K, Bateman K, Keefe GP, Walton J, Johnson W. (2002). The effect of treatment of clinical endometritis on reproductive performance in dairy cows. J. Dairy Sci. 85(9):2237-2249. https://doi.org/10.3168/jds.S0022-0302(02)74303-8

LeBlanc SJ, TF Duffield, KE Leslie, KG Bateman, GP Keefe, JS Walton, WH Johnson. (2002a). Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85:2223–2236. https://doi.org/10.3168/jds.S0022-0302(02)74302-6

LeBlanc SJ (2008). Postpartum uterine disease and dairy herd reproductive performance: a review. Vet. J. 176(1):102-114. https://doi.org/10.1016/j.tvjl.2007.12.019

Lee J-Y, Kim I-H (2006). Advancing parity is associated with high milk production at the cost of body condition and increased periparturient disorders in dairy herds. J. Vet. Sci. 7(2):161. https://doi.org/10.4142/jvs.2006.7.2.161

Leutert C, Von Krueger X, Plöntzke J, Heuwieser W (2012). Evaluation of vaginoscopy for the diagnosis of clinical endometritis in dairy cows. J. Dairy Sci. 95(1): 206-212 https://doi.org/10.3168/jds.2011-4603

McDougall S, Hussein H, Aberdein D, Buckle K, Roche J, Burke C, Mitchell M, Meier S. (2011). Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. Theriogenology. 76(2):229-240. https://doi.org/10.1016/j.theriogenology.2010.12.024

McNaughton A, Murray R (2009). Structure and function of the bovine fetomaternal unit in relation to the causes of retained fetal membranes. Vet. Rec. 165(21): 615-622. https://doi.org/10.1136/vr.165.21.615

Muneer M, Arshad M, Ahmad M, Ahmad I, Rauf A, Abbas S (1991). Antibiotic sensitivity of bacteria causing metritis in cows. Pakistan Vet. J.

Oltenacu P, Britt J, Braun R, Mellenberger R (1983). Relationships among type of parturition, type of discharge from genital tract, involution of cervix, and subsequent reproductive performance in Holstein cows. J. Dairy Sci. 66(3):612-619. https://doi.org/10.3168/jds.S0022-0302(83)81832-3

Opsomer G, Gröhn Y, Hertl J, Coryn M, Deluyker H, de Kruif A (2000). Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology. 53(4):841-857. https://doi.org/10.1016/S0093-691X(00)00234-X

Piersanti RL, Zimpel R, Molinari PC, Dickson MJ, Ma Z, Jeong KC, Santos JE, Sheldon IM, Bromfield JJ (2019). A model of clinical endometritis in Holstein heifers using pathogenic Escherichia coli and Trueperella pyogenes. J. Dairy Sci. 102(3):2686-2697. https://doi.org/10.3168/jds.2018-15595

Pleticha S, Drillich M, Heuwieser W. (2009). Evaluation of the Metricheck device and the gloved hand for the diagnosis of clinical endometritis in dairy cows. J. Dairy Sci. 92(11):5429-5435. https://doi.org/10.3168/jds.2009-2117

Runciman D, Anderson G, Malmo J. (2009). Comparison of two methods of detecting purulent vaginal discharge in postpartum dairy cows and effect of intrauterine cephapirin on reproductive performance. Australian Vet. J. 87(9):369-378. https://doi.org/10.1111/j.1751-0813.2009.00469.x

Runciman D, Anderson G, Malmo J, Davis G. (2008). Use of postpartum vaginoscopic (visual vaginal) examination of dairy cows for the diagnosis of endometritis and the association of endrometritis with reduced reproductive performance. Australian Vet. J. 86(6):205-213. https://doi.org/10.1111/j.1751-0813.2008.00301.x

Salasel B, Mokhtari A, Taktaz T. (2010). Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. Theriogenology. 74(7):1271-1278. https://doi.org/10.1016/j.theriogenology.2010.05.033

Šavc M, Duane M, O'Grady LE, Somers JR, Beltman ME. (2016). Uterine disease and its effect on subsequent reproductive performance of dairy cattle: a comparison of two cow-side diagnostic methods. Theriogenology. 86(8):1983-1988. https://doi.org/10.1016/j.theriogenology.2016.06.018

Sheldon IM. (2020). Diagnosing postpartum endometritis in dairy cattle. Vet. Rec. 186(3):88. https://doi.org/10.1136/vr.m222

Sheldon I, Bushnell M, Montgomery J, Rycroft A. (2004). Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. Vet. Rec. 155(13):383-387. https://doi.org/10.1136/vr.155.13.383

Sheldon I, Noakes D, Rycroft A, Pfeiffer D, Dobson H. (2002). Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction-Cambridge- 123(6):837-845. https://doi.org/10.1530/rep.0.1230837

Sheldon IM. (2004). The postpartum uterus. Vet. Clin.: Food Anim. Pract. 20(3):569-591. https://doi.org/10.1016/j.cvfa.2004.06.008

Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth H-J (2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol. Reprod. 81(6):1025-1032. https://doi.org/10.1095/biolreprod.109.077370

Sheldon IM, Cronin JG, Bromfield JJ. (2019). Tolerance and innate immunity shape the development of postpartum uterine disease and the impact of endometritis in dairy cattle. Annu. Rev. Anim. Biosci. 7:361-384. https://doi.org/10.1146/annurev-animal-020518-115227

Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO (2006). Defining postpartum uterine disease in cattle. Theriogenology. 65(8):1516-1530. https://doi.org/10.1016/j.theriogenology.2005.08.021

Singh J, Murray R, Mshelia G, Woldehiwet Z. (2008). The immune status of the bovine uterus during the peripartum period. Vet. J. 175(3):301-309. https://doi.org/10.1016/j.tvjl.2007.02.003

Studer E (1981). Evaluation of the post partum reproductive tract in the bovine;p 113-125.

Williams EJ, Fischer DP, Pfeiffer DU, England GC, Noakes DE, Dobson H, Sheldon IM. (2005). Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology. 63(1):102-117. https://doi.org/10.1016/j.theriogenology.2004.03.017

Zemjanis R. (1980). Repeat breeding or conception failure in cattle. Current therapy in theriogenology. 1:209.