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Research article

Identification of prevalence and antibiotic resistance property as a basis for establishing an efficient treatment of bacteria causing mastitis in beef cows

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Abstract

The purpose of the study was to examine the occurrence of metritis on reproduction of beef cows. A total of 2,962 cows were examined, and samples were collected from those displaying clinical symptoms of bovine metritis for bacterial analysis. Bacterial species identification was performed using culture and PCR techniques. The findings revealed that bovine metritis affected 5.5% of the reproductive cow population with the presence of *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. at the rates of 87.7%, 61.1%, 59.9% and 17.3%, respectively. The results also demonstrated that *Streptococcus* spp. was the most prevalent group, while *Salmonella* spp. had the lowest prevalence across different breeds and litters. However, in the Limousin crossbred cows, the infection rate of *E. coli* surpassed that of the other bacteria and a significantly higher infection rate for *E. coli* was observed in litter 4 and litter ≥6 compared to the others. The disk diffusion method was utilized to assess antibiotic resistance patterns of the isolated bacteria. Among the bacteria, marbofloxacin exhibited the lowest prevalence of antibiotic resistance (16.9%), while doxycycline had the highest prevalence (82.2%). All cows recovered from the disease within 3-5 days of treatment, and all of them resumed estrus in the subsequent reproductive cycle. The pregnancy rate ranged from 66.7% to 80.0% for the first insemination in the next reproductive cycle.

Keywords: Bovine metritis, *Escherichia coli*, *S. aureus*, *Salmonella* spp., *Streptococcus* spp.

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INTRODUCTION

Bovine metritis is a common disease in reproductive cows, and the consequences often lead to reproductive disorders, delayed birth, infertility and loss of milk (Sheldon and Dobson, 2004; Le Blanc, 2008). Hansen et al. (2013) demonstrated that the development of metritis or endometritis in some cows might be caused by the exposure to a high amount of bacteria during the calving process than others. Further, *Staphylococcus* spp., *Streptococcus* spp., and *Escherichia coli* (*E. coli)* were widely accepted as the dominant bacteria in the uterus. Among them, *E. coli* was unanimously regarded as the most predominant bacteria (Baishya et al., 1998; Sheldon et al., 2004; Williams et al., 2005; Wagener et al., 2014; Abreham et al., 2017). These bacteria were found in the uterine environment of cows after calving in more than 95% of cases (Sheldon and Dobson, 2004). In particular, the bacteria are detected in the uterus from the first week after the birth of 90% of reproduction cows (Sheldon et al., 2002). Usually, cows are susceptible to bacteria such as *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., etc. These bacteria enter and colonise in the genital tract, and under favorable conditions, they will proliferate to a sufficient quantity to cause diseases in cattle.

Recently, Madhumeet et al. (2018) revealed that the bacteria were found in 22 out of 24 endometritis cows (91.7%). Among them, the most frequent were *Streptococcus* spp. and *E. coli* (20.8% each), followed by *S. aureus* (12.5%), and none of *Salmonella* spp. was found. A study by Moges et al. (2013) revealed the presence of *E. coli*, *Streptococcus* spp., *Staphylococcus aureus (S. aureus)*, and some other bacteria in the inflammatory fluid of metritis cows. In addition, Yang et al. (2016) revealed that cow uterine could be damaged easily during the parturition process and the regular existence of *E. coli* was triggering and prolonging the inflammation of the uterus. This phenomenon leads to high economic losses due to prolonged calving cycles, lower conception rates, and increased elimination rates. As a consequence, bovine metritis can result in economic losses of farmers, and it is also one of the reasons hindering the development of the herd in terms of both quantity and quality (Bromfield et al., 2015).

According to Sheldon et al. (2006), the damage to reproductive ability depends on the characteristics of the bacteria population, severity of infection, and duration of inflammation. Therefore, antibiotics were used commonly for the treatment of the bacteria causing metritis to shorten the inflammation stage (Singh et al., 2014). However, the excessive and indiscriminate use of antibiotics to treat bacterial infections in the uterus has led to the emergence of antibiotic-resistant strains. Indeed, antibiotic overuse has resulted in multiple drug resistance and increased the proportion of mortality (Gurunathan, 2015; Yah and Simate, 2015). Furthermore, this increased risk of antimicrobial resistance leads to less effective conventional treatments. The focus of the current study is to identify the causative bacteria by isolating them from the inflammatory fluid in the uterus of beef cows, which enables the evaluation of antibiotic resistance patterns and the proposal of effective treaments.

MATERIALS AND METHODS

Collecting and preserving samples

Samples of uterine swab fluids were collected from metritis-affected beef cows in Tien Giang province, Vietnam, for the purpose of isolating and identifying bacteria. A total of 2,962 cows from different breeds and litters were investigated and samples of suspected cows were collected during estrous. All inflammatory fluid samples were collected before antibiotic administration. The sample collection process was applied as follows: (1) wiped the vulva area with a tissue and disinfected with 70° alcohols, (2) inserted a sterilized colposcope into the cow's vagina, (3) pushed a sterilized swab through these tools to collect pus. The swabs were stored in a test tube containing the storage medium and later the samples were refrigerated at 2°C to 8°C until bacteria isolation and identification (Moriarty et al., 2008; Bicalho et al., 2017).

Bacterial isolation

In the first step after collection, the samples were proliferated in Nutrient Broth (NB) and NB supplemented with 5% sheep blood. The isolation of specific bacteria was conducted using a specialized medium, such as *E. coli* on Tryptone Bile X-glucuronide agar (TBX, Blake et al., 2003), *Salmonella* spp. on Mannitol Lysine Crystal Violet Brilliant green (MLCB)/Xylose Lysine Desoxycholate (XLD) (Yoshihiko et al., 2000), *S. aureus* on Mannitol Salt Agar (MSA) (Kateete et al., 2010), and *Streptococcus* spp. on Selective Strep Agar with 5% Sheep Blood (Jorgensen et al., 2015). When typical colonies were obtained, the selected colonies were cultured on Trypticase Soy Agar (TSA) medium for a second proliferation. The isolated bacteria including *Salmonella* spp., *S. aureus*, and *Streptococcus* spp. were then re-confirmed by Polymerase Chain Reaction (PCR) technique.

Identification of *Salmonella* spp., *S. aureus*, and *Streptococcus spp.* by PCR technique

Bacterial DNA was extracted using the heat shock method of Soumet et al. (1994). The primers used to amplify the specific genes of *S. aureus* (Nuc-F 5'-GCGATTGATGGTGATACGGTT-3'; Nuc-R 5'-AGCCAA GCCTTGACGAACTAAAGC-3'), *Salmonella* spp. (invA-F 5'-GTGAAA TTATCGCCACGTTCGGGCAA-3'; invA-R 5'-TCATCGCACCGTCAA AGGAACC-3'), and *Streptococcus* spp. (Str-F 5'-GTACAGTTGCTTCAG GACGTATC-3'; Str-R 5'-ACGTTCGATTTCATCACGTTG-3') were picked up from Brakstad et al. (1992), Zahraei et al. (2005), and Irmler et al. (2009). PCR products were electrophoresed on 1.5% agarose gel at 110 V, 400 mA for 15 minutes and the images were visualized under a UV light.

Antibiogram method

Antibiogram was performed based on the method of Kirby-Bauer, (1996) and the sensitivity of antibiotics resistance bacteria was determined by the zone of inhibition around the antibiotic-impregnated disks (CLSI, 2008). The antibiotics were ampicillin 10 µg (Am), colistin 10 µg (Co), doxycycline 30 µg (Dx), gentamicin 10 µg (Ge), ceftiofur 30 µg (FUR30), marbofloxacin 5 µg (MAR5), and florfenicol 30 µg (FFC30). After being taken out from the freezer,antibiotics must be thawed for 15 to 30 minutes before administration. Purely cultured colonies were placed in a tube containing 9 ml of 0.9% NaCl, and the mixture was adjusted for a concentration of 108 CFU/ml. Spread 100 µl of the above mixture evenly on the surface of the Mueller-Hinton Agar (MHA) and placed the antibiotic-impregnated disks in full contact with the agar surface (minimum distance 24 mm) followed by incubating at 37°C for 16-24 hours. In case of Streptococcus spp., the MHA was added with 5% sheep blood and incubated for 24 hours under anaerobic conditions at 37°C. The diameters of inhibition zones (mm) were measured and compared with CLSI (2010, 2016), EUCAST (2017), and BSAC (2013) to determine whether the antibiotic resistance profile was sensitive (S), intermediate (I), or resistance (R).

Treatment regimen

Two highly sensitive antibiotics were chosen and combined with additional supplements to enhance their effectiveness. Regimen A included marbofloxacin along with 0.2% rivanol, ADE vitamin, and the anti- inflammatory dexamethasone. Regimen B involved the intramuscular administration of gentamycin combined with prostaglandin, ADE vitamin, and the anti-inflammatory dexamethasone. A total of 60 cows (30 animals / regimen) with metritis were treated using these two regimens for a duration of 3-5 days. Recovery from metritis was confirmed based on specific indicators including the absence of inflammatory discharge from the vagina after the treatment period and no inflammatory fluid leaking from the uterus during dilation in the subsequent breeding cycle. Subsequently, pregnancy was confirmed by evaluating signs such as the absence of estrus in the following cycle, ultrasound confirmation at 32 days, and rectal examination at 60 days.

Statistical analysis

Data were collected, coded, and processed using Microsoft excel 2016. The prevalence of pathogenic bacteria groups was calculated by dividing the cows that were positive for the bacteria by the total number of cows. Statistical differences in the prevalence of bacteria were tested using the Chi-square and Fisher's exact test in the Minitab (version 16.0) software.

RESULTS

Prevalence of bovine metritis

In reproductive beef cows, the prevalence of bovine metritis was determined to be 162 out of 2,962 individuals, accounting for 5.5% of the total. *Streptococcus* spp. (87.7%), *S. aureus* (61.1%), and *E. coli* (59.9%) were the most commonly identified bacteria in the uterine fluid of 162 affected cows. Non-permanent bacteria in bovine metritis *Salmonella* spp. constituted 17.3% of the whole (Figure 1).

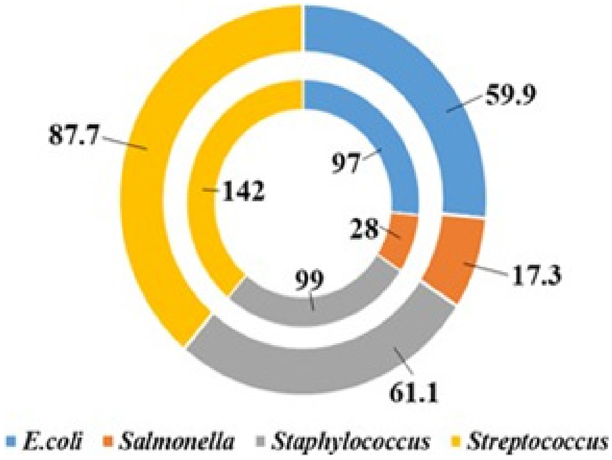

Figure 1 Prevalence (%) of bacteria groups in clinical samples

Bacteria prevalence by breed

The findings of bacterial isolation from samples of inflammatory fluid by breeds are shown in Table 1. Except for the BBB crossbred cattle, the prevalence of bacteria groups detected in other breeds differs statistically ($P \leq 0.05$). In detail, *Streptococcus* spp. ranged in incidence from 82.2% to 95.8%, followed by *Staphylococcus* spp. (52.9%-71.4%), *E. coli* (42.1%-100%), and *Salmonella* spp. (0.0%-29.8%).

Table 1 Prevalence (%) of metritis-associated bacteria in different cattle breeds

Breed	Bacteria				P
	E. coli	*Sal*	*Sta*	*Strep*	
Crossbred BBB (n=8)	62.5	37.5	75.0	75.0	0.362
Crossbred Brahman (n=24)	66.7^{b}	29.2^{c}	54.2^{bc}	95.8^{a}	0.000
Crossbred Charolais (n=35)	68.6^{ab}	20.0^{c}	57.1^{b}	85.7^{a}	0.000
Crossbred Sindhi (n=34)	44.1^{b}	8.82^{c}	52.9^{b}	88.2^{a}	0.000
Crossbred Limousin (n=7)	100^{a}	28.6^{b}	71.4^{ab}	85.7^{a}	0.020
Crossbred Red Angus (n=35)	62.9^{a}	17.1^{b}	71.4^{a}	82.9^{a}	0.000
Local cattle (n=19)	42.1^{b}	0.0^{c}	63.2^{b}	94.7^{a}	0.000

^{a,b,c}: Values within a row with different superscripts are significantly different ($P \leq 0.05$); *Sal: Salmonella, Sta: Staphylococcus, Strep: Streptococcus*

Bacteria coinfection by breed

The analysis of the bacteria examined revealed that there was no significant difference in coinfection rates among the different breed groups. However, a notable distinction was observed in the coinfection of bacteria groups in most specific breeds, except for Brahman and Limousin crossbred cows. Table 2 displays the findings indicating that the highest proportion of bacterial infections was observed in BBB, Charolais, Red Angus crossbred and local cows, with two groups of bacteria being the most prevalent.

Table 2 Bacteria coinfection by breed

Breed	No. of bacteria group in the coinfection				P
	1	2	3	4	
Crossbred BBB (n=8)	0.0[b]	50.0[a]	50.0[a]	0.0[b]	0.014
Crossbred Brahman (n=24)	16.7	37.5	29.2	16.7	0.261
Crossbred Charolais (n=35)	17.1[b]	40.0[a]	37.1[ab]	5.71[c]	0.002
Crossbred Sindhi (n=34)	32.4[a]	41.2[a]	26.5[a]	0.0[b]	0.001
Crossbred Limousin (n=7)	0.0	28.6	57.1	14.3	0.083
Crossbred Red Angus (n=35)	8.6[b]	57.1[a]	25.7[b]	8.6[b]	0.000
Local cattle (n=19)	21.1[b]	57.9[a]	21.1[b]	0.0[c]	0.001

[a,b,c] : Values within a row with different superscripts are significantly different (P≤0.05)
1. group: single infection of E. coli or Salmonella or Staphylococcus or Streptococcus
2. groups: coinfection of two bacteria
3. groups: coinfection of three bacteria
4. groups: coinfection of four bacteria

Bacteria prevalence by litter

Table 3 presents the results of bacterial isolation from inflammatory fluid obtained from metritis cows, categorized by litter. The findings indicated that the presence of *E. coli*, *Salmonella* spp., *Streptococcus* spp., and *S. aureus* in clinical samples varied significantly across different litters. Among them, the highest prevalence was *Streptococcus* spp. (75-100%), followed by *S. aureus* (47.4-70.0%), *E. coli* (45.0-68.4%), and the lowest was *Salmonella* spp. (5.3-66.7%). At litter ≥6, the highest prevalence was still *Streptococcus* spp. (89.5%), but the presence of *E. coli* increased (68.4%) compared to *S. aureus* (47.4%), and the lowest was still *Salmonella* spp. (5.3%). It can be seen that cows in litters 1, 2, and 3 have higher prevalence of bacteria causing metritis than cows in litters 4 and ≥5.

Table 3 Prevalence of bacteria groups in metritis cows by litter

Parity	Bacteria				P
	E. coli	*Sal*	*Sta*	*Strep*	
Heifers (n=6)	66.7	66.7	83.3	83.3	0.828
Litter 1 (n=20)	45.0[b]	20.0[c]	70.0[bc]	100[a]	0.000
Litter 2 (n=44)	54.4[b]	20.5[c]	63.6[b]	90.9[a]	0.000
Litter 3 (n=36)	66.7[b]	13.9[c]	63.9[b]	86.1[a]	0.000
Litter 4 (n=25)	68.0[a]	12.0[b]	56.0[a]	80.0[a]	0.000
Litter 5 (n=12)	50.0[ab]	16.7[b]	50.0[ab]	75.0[a]	0.041
≥ Litter 6 (n=19)	68.4[b]	5.26[b]	47.4[b]	89.5[a]	0.000

[a,b,c]: Values within a row with different superscripts are significantly different (P≤0.05);
Sal: Salmonella, Sta: Staphylococcus, Strep: Streptococcus

Bacteria coinfection by litter

The results on coinfection rates of bacterial groups by litter are presented in Table 4. Except for the 5th litter, a significant difference was observed among the groups. In heifers, the coinfection was recorded only by 2 and 4 bacteria with a rate of 50%. For the remaining litters, coinfection by two bacteria groups was highest, followed by three groups, the lowest was one group and four groups.

Table 4 Bacteria coinfection by litter

Litter	No. of bacteria group in the coinfection				P
	1	2	3	4	
Heifers (n=6)	0 (0.00)[b]	3 (50.0)[a]	0 (0.00)[b]	3 (50.0)[a]	0.046
Litter 1 (n=20)	1 (5.00)[b]	12 (60.0)[a]	6 (30.0)[a]	1 (5.00)[b]	0.000
Litter 2 (n=44)	7 (15.9)[b]	19 (43.2)[a]	16 (36.4)[a]	2 (4.55)[b]	0.000
Litter 3 (n=36)	5 (13.9)[b]	17 (47.2)[a]	12 (33.3)[a]	2 (5.56)[b]	0.000
Litter 4 (n=25)	6 (24.0)[a]	10 (40.0)[a]	8 (32.0)[a]	1 (4.00)[b]	0.023
Litter 5 (n=12)	5 (41.7)	4 (33.3)	2 (16.7)	1 (8.33)	0.217
≥ Litter 6 (n=19)	4 (21.1)[a]	9 (47.4)[a]	6 (31.6)[a]	0 (0.00)[b]	0.007

[a,b,c] : Values within a row with different superscripts are significantly different ($P \leq 0.05$)

1. group: single infection of E. coli or Salmonella or Staphylococcus or Streptococcus

2. groups: coinfection of two bacterial

3. groups: coinfection of three bacterial

4. groups: coinfection of four bacterial

Antibiotic resistance patterns of bacterial groups

The findings from Table 5 indicate that the bacteria in the inflammatory fluid of cows display the highest resistance rate (66.6%) to Co, which is significantly different from the resistance rates observed for other antibiotics ($P<0.001$). It is followed by FUR30 (57.9%), Am (45.2%), DX (40.7%), FFC30 (29.2%), Ge (24.9%) and the lowest MAR5 (16.9) %). The above results showed that the bacteria strains were highly resistant to Co, whereas these bacteria were relatively sensitive to MAR5. Furthermore, a notable and statistically significant difference was observed in the antibiotic resistance patterns among the various groups of bacteria, except for MAR5 and FUR30. However, it is worth noting that the resistance of bacteria groups to FUR30 was relatively high (36.7-62.6%). Conversely, the resistance of bacteria groups to MAR5 was relatively low (6.7-19.7%, and it was the lowest among most bacteria groups. Therefore, this antibiotic group should be considered more in the treatment process.

Table 5 Antibiotic resistance patterns of infected bacteria

Antibiotic	Bacteria					P
	E. coli (n=115)	*Sal* (n=30)	*Sta* (n=117)	*Strep* (n=163)	Total (n=425)	
Am	$^{C}33.9^{c}$	$^{B}56.7^{a}$	$^{AB}45.3^{b}$	$^{C}50.9^{a}$	$^{C}45.2$	0.022
Dx	$^{D}13.0^{c}$	$^{BC}50.0^{b}$	$^{E}7.7^{c}$	$^{A}82.2^{a}$	$^{C}40.7$	0.000
Ge	$^{C}31.3^{a}$	$^{C}26.7^{a}$	$^{CD}29.9^{a}$	$^{D}16.6^{b}$	$^{D}124.9$	0.017
FUR30	$^{B}57.4$	$^{BC}36.7$	$^{A}57.3$	$^{B}62.6$	$^{B}57.9$	0.071
Co	$^{A}79.1^{a}$	$^{A}86.7^{a}$	$^{C}31.6^{b}$	$^{A}79.1^{a}$	$^{A}66.6$	0.000
FFC30	$^{C}31.3^{a}$	$^{BC}46.7^{a}$	$^{BC}34.2^{a}$	$^{D}20.9^{b}$	$^{D}29.2$	0.009
MAR5	$^{D}13.9$	$^{D}6.7$	$^{D}19.7$	$^{D}19.0$	$^{E}16.9$	0.249
P	0.000	0.000	0.000	0.000	0.000	

a, b, c : Values within a row with different superscripts are significantly different (P≤0.05)
A, B, C, D, E: Values within a column with different superscripts are significantly different (P≤0.05)
Sal: Salmonella, Sta: Staphylococcus, Strep: Streptococcus, Am: Ampicillin, FUR30: Ceftiofur, Co: Colistin, Dx: Doxycycline, FFC30: Florfenicol, Ge: Getamicin, MAR5: Marbofloxacin.

For Am antibiotic, *Salmonella* is the most resistant bacteria (56.7%), followed by *Streptococcus* spp. (50.9%), *Staphylococcus* spp. (45.3%), and the lowest *E. coli* (33.9%). Similarly, for Co and FFC30 antibiotics, *Salmonella* spp. showed the highest resistance, the lowest resistance was *Staphylococcus* spp. (Co) and *Streptococcus* spp. (FFC30). For the Dx antibiotic, the highest resistance was *Streptococcus* spp., followed by *Salmonella* spp., and the lowest was *Staphylococcus* spp. Antibiotics Ge, *E. coli* showed the highest resistance rate, followed by *Staphylococcus* spp., and the lowest was *Streptococcus* spp. In the present study, *E. coli* was resistant to Ge at 31.3%, while the FUR30 resistance rate was 57.4%.

Regimens and results of treatment for bovine metritis

Based on the results of the antibiogram, Marbofloxacin and Gentamicin were selected for treatment experiment. The results in Table 6 indicate that both treatment regimens can stop 100% of inflammation in treated cows after 3-5 days of treatment. This result confirmed the effectiveness of using MAR5 and Ge for metritis treatment in cows. After recovery from the metritis, 100% of cows were re-estrus. Importantly, there was no inflammatory fluid in the uterus of 60 treated cows in the next reproductive cycle. In addition, the pregnancy rate was high (from 66.7% to 80.0%) with two regimens when cows were estrous and inseminated in the next reproductive cycle.

Table 6 The recovery of metritis cows in two treatment regimens

Treatment regimen	Treatment number	Healing		No. of estrus returns		No. of cows pregnant	
		Head	Rate (%)	Head	Rate (%)	Head	Rate (%)
1	30	30	100	30	100	24	80.0
2	30	30	100	30	100	20	66.7

DISCUSSION

Prevalence of bovine metritis and the identification of bacteria

The objective of this study was to examine the presence of commonly encountered bacteria responsible for metritis in beef cows and evaluate their susceptibility to commonly used antibiotics for treating metritis in cows. The findings from the antibiotic resistance testing on the four groups of bacteria revealed significant resistance to Co and FUR30. However, a majority of the bacteria examined in this study exhibited high sensitivity to MAR5. Therefore, cows affected by metritis caused by the aforementioned four groups of bacteria can be effectively treated using MAR5 antibiotics.

According to Sheldon et al. (2006), approximately 15% of dairy cows showed clinical signs of chronic uterine disease three weeks after calving. This phenomenon is called clinical endometritis and is characterized by the presence of pus in the uterus, which can often be detected in the vagina. The current findings (Figure 1) showed a lower prevalence of bovine metritis (5.5%) compared to the previous study (7.2%) conducted in Tien Giang by Dong (2016). The frequency of bovine metritis varied based on location and historical period. Within Vietnam, Nha and Anh (2008) reported a low metritis prevalence of 3.2% in reproductive cows in Quang Nam province. Duong (2011) reported a bovine metritis prevalence of 5.6% in reproductive cows in Nghe An province while Kien (2012) discovered a high bovine metritis prevalence (22.9%) in reproductive cows in the Ba Vi district of Hanoi city. The aforementioned outcomes validate the relatively low occurrence of bovine metritis observed in this study. This could potentially be attributed to the progress made in artificial insemination techniques for reproductive cows, which has led to enhanced safety measures and a reduced risk of genital infections like metritis. Moreover, it was also indicated that a majority of cows received vaccinations, the farming conditions were satisfactory, and the rearing environment was deemed acceptable. These combined factors likely contribute to a decreased prevalence of metritis among cows in the region.

The findings of the present study (Figure 3) are similar to those of Tien (2006) which focused on the indicators of bovine metritis in dairy cows in some districts of Hanoi and Bac Ninh. These authors indicated that the dominant bacteria population in the inflammatory fluid were *E. coli*, *S. aureus*, *Streptococcus* spp. with a prevalence of 100%, unclassified bacteria accounted for 76.0% and non-permanent bacteria are *Salmonella* spp. constituting 28.0%. However, in the current work (Table 1), the highest prevalence of *E. coli* was observed only in the Limousin crossbred cattle. *Streptococcus* spp. was the dominant bacteria in the inflammatory fluid samples. Extraordinarily, the persistence of microorganisms in utero of the cow population changed with time, highest in the first two weeks after calving (more than 90%), followed by the period from day 16 to day 30 (78%), 31 to 45 (50%), and 45 and 60 (9%) (Smith and Risco, 2002). Therefore, monitoring the bacteria population in utero of cows should be considered a regular activity to control bovine metritis. In our study, cows in litters 1, 2, and 3 have a higher prevalence of bacteria causing metritis than cows in litters 4 and ≥5 (Table 3). This tendency may be explained by the immature sexual characteristics of cows in the first calving, which might cause difficulty in mating. In addition, the pelvis may not adapt perfectly to the parturition process, thereby causing injury endometrial leads to inflammation. This hypothesis was supported by the study of Lohuis et al. (1994) in which *E. coli*, *Staphylococcus* spp., and *Streptococcus* spp. were isolated regularly from cows with residual placenta and cows with clinical endometritis. Furthermore, Huszenicza et al. (1999) confirmed that *E. coli* was found in the uterus of cows with the postpartum disease. In addition, a significant role of *E. coli* in the metritis-endometritis syndrome complex was revealed in previous studies (Williams et al., 2005; Donofrio et al., 2008). *E. coli* can infect and be persistent in the epithelial layer of the endometrium leading to the high prevalence of *E. coli* infection. Moreover, the interaction between *E. coli* and α-*Streptococcus* in bovines at week 7 was revealed previously (Huszenicza et al., 1999). The coincidence of bacterial persistence and the immaturity of the reproductive tract was the critical factor leading to the high prevalence of bovine metritis in the early litters of the reproductive period in cows. Therefore, carefully monitoring during the first reproductive cycle is necessary for early countermeasures to reduce the damage of the infection.

The coinfection of bacteria in bovine metritis is common in the early postpartum period, occurring in the first two weeks postpartum (days 4-10), and characterized by a large amount of vaginal discharge with a foul, reddish-brown, watery with some necrotic debris in the uterus. In the first half of this period, the uterine wall became thin. In the next few days, a small amount of foul-smelling, purulent uterine was discharged and thickened uterine wall (bloody) (Sheldon and Dobson, 2004; Sheldon et al., 2006). The study of Ghanem et al. (2014) mentioned that more than one bacteria species was found in the uterus of some cows at week 5. Interestingly, some cows that were negative for bacterial infection at week five developed an infection at week 7. These results may be related to the interaction of uterine immune response and the ability to clear the infection of the uterus. This result was consistent with a study by Huszenicza et al. (1999), who discovered that metritis pathogens could also be isolated from the uterus of cows without clinical signs of endometritis. On the other hand, Bacha and Regassa (2010) reported that most cows negative for endometritis at week 4 were also negative at week 8. In addition, a higher proportion of endometritis cows in week 4 compared to week 8 was recorded. In general, the development of many bacteria species at the same time was common in bovine metritis. The interaction among bacteria to obtain a synergistic effect aggravated clinical signs that were reported previously. Based on the current study, coinfection by 2 and 3 groups of bacteria was the most common, meaning that treatments should consider the characteristics of coinfected bacteria to optimize their efficiency.

The antibiotic resistance profile of bacterial groups

To avoid failure of treatments due to antibiotic resistance, the previous study tried to apply other methods such as the mixture of lauric acid with acetic and lactic acid (Pangprasit et al., 2020) or ethanolic extract of Morus alba leaves (Phengvongsone et al., 2022) for bovine metritis treatments. However, the success of the above treatments was reported *in vitro* only. Therefore, antibiotics are still considered as an effective counter to control bacterial infection or prevent the progression of the disease. Antibiotic compounds such as tetracyclines, sulfonamides, aminoglycosides, β-lactams, and cephalosporins have been used together or alone to treat postpartum metritis. The massive use of antibacterial agents in dairy cows may lead to bacterial resistance strains (El-Khadrawy et al., 2015). The research of Benko et al. (2015) showed that *E. coli* and *Streptococcus* spp. have less resistance to marbofloxacin (ranging from 2.3% to 7.4%), while *Staphylococcus* was not resistant to this antibiotic. In this study, the authors also found a difference in resistance to the same antibiotic of the same bacteria group. Specifically, the gentamycin resistance of β-hemolytic *Streptococcus* was 82.2% while that of *Streptococcus* spp. was 48.2%. Similarly, the gentamycin resistance of haemolytic *E. coli* was 16.7%, and non-haemolytic *E. coli* was up to 41.6%. For the marbofloxacin, the authors found a similar trend, but the resistance rate to this antibiotic was low (0%-7.4%). According to the ASTS report (1997-1998), in Vietnam, many bacterial strains such as *E. coli*, Enterobacter, *Shigella flexneri*, *Salmonella typhi*, *S. aureus* were resistant to doxycycline (Vietnam Ministry of Health, 2018). Research by Moges et al. (2013) indicated the gentamicin resistance of *E. coli*, *Staphylococcus* spp, and *Streptococcus* spp. isolated from metritis cows was up to 33.3% and higher than the present results (24.9%).

When evaluating the antibiotic sensitivity of bacterial groups, Madhumeet et al. (2018) showed that all isolates of *S. aureus* were resistant to ampicillin; 40% of *E. coli* to gentamycin. Meanwhile, *Streptococcus* spp. were sensitive to all tested antibiotics. According to other studies, *E. coli* isolated from utero was sensitive to gentamycin (Noakes et al., 1989) or ceftiofur (Drillich et al., 2001). In the present study (Table 5), *E. coli* were resistant to gentamycin at 31.3%, while the ceftiofur resistance rate was 57.4%. The above results showed that antibiotic resistance of bacteria was different by location and objects. Particularly, the antibiotic resistance of bacteria isolated in the tropical region may vary, meaning that the selection of appropriate antibiotics for treatment highly depended on the antibiogram of the current bacteria. This phenomenon was a limiting factor that reduced the effectiveness of popular antibiotic treatment regimens used in these regions.

Regimens and results of treatment for bovine metritis

The major reason for the low conception rate was that cows have uterine inflammation, giving rise to reduced reproductive performance and an increased number of insemination times per conception. Besides, it also delays the first day of artificial insemination and prolongs the day of calving to the time of the next conception, thereby reducing conception rates in cows (Le Blanc, 2008). Endometritis was associated with increased days of reproductive maturation and low conception rates in cows (Sheldon and Dobson, 2004). Therefore, minimizing the burden due to prolonging the inflammation is of utmost importance in treatment. The current regimens show high efficacy for bovine metritis treatment with the recovery reaching 100%. However, the pregnancy rate did not reach the appropriate level in the two regimens. The lower conception rate of cows in regimen two compared to cows in regimen one might relate to a higher resistance rate against gentamicin than marbofloxacin. In addition, metritis has influenced the uterus even after cows had recovered. Cows recovering from metritis might lack the optimal conditions for sperm transport and storage, oocyte maturation and ovulation, zygote development, implantation, and growth of embryo (Gilbert, 2011). Therefore, asymptomatic metritis significantly reduces the productivity of cow's milk (Sheldon et al., 2006). What can be implied from these is that controlling bovine metritis at early stages is better than using antibiotics to treat infected cows.

CONCLUSIONS

This study revealed the status of bovine metritis in reproductive cows in Tien Giang province. The prevalence of bovine metritis in reproductive cows was low at 5.5%. In metritis cows, permanent bacteria such as *E. coli*, *S. aureus*, and *Streptococcus* spp., were discovered. Besides, *Salmonella* spp. was confirmed as non-permanent bacteria in the metritis cow population. In general, the isolated bacteria exhibited a high resistance to colistin and were sensitive to marbofloxacin. When applied for treatment, the regimen with marbofloxacin showed a high recovery (100%) and a high number of pregnancies after treatment (80%).

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AUTHOR CONTRIBUTIONS

Tran Hoang Diep; Investigation, laboratory analysis, manuscript preparation.

Nguyen Trong Ngu; Conceptualization and design the experiment, investigation, supervision, editing and finalization.

CONFLICT OF INTEREST

We have no conflict of interest.

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