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Original article

Prevalence of isolated bacteria from clinical endometritis uterine and antimicrobial susceptibility in postpartum dairy cows

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Abstract The objectives of this study were to determine the prevalence of bacteria from endometritis postpartum dairy cows and determination of their antimicrobial susceptibility in dairy cows with 30-90 days postpartum. Holstein-Frisian cows, lactation 1-8, 30-90 days postpartum in small holder dairy farms were diagnosed as clinical endometritis by Metricheck™ device and collected the discharge by uterine swabbing with AI technique, aseptically. Samples were examined for aerobic bacterial identification and antimicrobial sensitivity. Fifty-six cows from 30 small holder dairy farms were diagnosed as clinical endometritis and had bacterial cultured. Most of cows (82.76%) had vaginal discharge score equal 2. Twenty cows were recorded for retained fetal membrane after calving. Isolates include gram-positive bacteria (n=58) and gram-negative bacteria (n=42). The most frequency isolates of bacteria were as follow: *E. coli* (24%), *Corynebacterium* spp. (18%), *A. pyogenes* (14%), *Staphylococcus* spp. (11%) and *Streptococcus* spp. (9%). From all 9 antimicrobial agents used in the study, gentamicin was the most sensitive antibiotics and oxytetracycline was the most resistant for both gram-positive and gram- negative bacteria. This finding may be the result of that oxytetracycline was routinely used in dairy farms in this study area. In conclusion, this study provided an update information of bacteria causing endometritis and their status of antibiotic resistance. Such information is useful for therapeutic treatment planning and antibiotic control usage. **Chiang Mai Veterinary Journal 2013; 11(3): 237-245**

Keywords: endometritis, bacteria, antimicrobial susceptibility, dairy cows

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Introduction

Bacterial contamination of the uterine lumen after parturition is common in dairy cattle (Miller et al., 2007). During and after parturition of dairy cows, a wide range of microorganisms ascend from the environment, invade the birth canal and colonize the uterus (Silva et al., 2008). Most healthy cows are able to spontaneously clear this contamination in the next 2–4 weeks (LeBlanc et al., 2002). However, the persistence of pathogenic bacteria will lead to the establishment of clinical metritis or clinical endometritis. Postpartum uterine infection is one of the most important disease (Fox et al., 2002; Sriskandan et al., 2000) causes economic losses in dairy cows due to a longer calving interval, costs of extra services and treatment, and increased culling rate (Drillich et al., 2005; Gilbert et al., 2005).

The presence of pathogenic bacteria in the uterus causes inflammation and histological lesion of endometrium. The clinical signs of uterine infection depend on number and virulence of the microorganism present and the conditions of the uterus, and its inherent defense mechanism. The pathogenic bacteria that are frequently isolated from endometritis cases are *A. pyogenes* and *E. coli*. Common bacteria isolated from cows with acute metritis and chronic endometritis are *Arcanobacterium pyogenes*, *Prevotella* spp., *Fusobacterium necrophorum* and *Escherichia coli* (Lewis, 1997; Smith et al., 1998; Overton et al., 2003; Sheldon and Dobson, 2004; Sheldon et al., 2006). Other studies also found *Staphylococcus* spp., *Streptococcus* spp. or non-*E.coli* aerobic gram-negative rods (Silva and Lobato, 1998; Kaczmarowski et al., 2004; Jadon et al., 2005).

The definitive diagnosis of endometritis is made on the basis of histological examination of endometrium biopsies (Cohen and Green, 2004). However, this technique is costly and time consuming, not clinically accessible in most situations (Sheldon et al., 2006). Hence, the examination of the contents of the vagina for presence of pus is proposed. Normal discharge from postpartum cows range in color from dark brown to red or white and usually should not be considered abnormal unless the fluid is malodorous or other aberrant clinical signs are found (LeBlanc et al., 2002). In contrast, the clinical signs of endometritis are the presence of a white or whitish-yellow mucopurulent vaginal discharge. The volume of the discharge is variable, but frequently increases at the time of estrus when the cervix dilates and there is abundant vaginal mucus. The cow rarely shows any signs of systemic illness (Jost et al., 2002).

Antibiotics therapy is a common treatment for metritis (Azawi, 2008). A variety of antimicrobial agents, administered by intrauterine infusion or parenteral injection, are normally used to treat uterine infection (Gustafsson, 1984). To choose an appropriate antimicrobial agent for endometritis treatment, it is essential to know the susceptibility of the pathogen to antibiotics. Thus, there should be information about drug sensitivity for metritis treatment because currently antimicrobial resistance in pathogenic bacteria has become a common problem.

In Thailand, the reports about endometritis in dairy cows, bacterial findings and antimicrobial susceptibility were very few and not up to date. The most recent studies were performed for 10-30 years. Currently, the new types of antibiotics are available. Thus, the using of antibiotic may be different from the past. It is essential to determine the current status of pathogens causing endometritis and their antibiotic resistance profile in order to control and treat the disease. The objectives of this study were to determine the prevalence of bacteria in abnormal uterine discharge and antimicrobial sensitivity profile in dairy cows with 30-90 days postpartum.

Materials and methods

Animals

The study was conducted between May and December 2012 on small holder dairy farms in Chiangmai and Lamphun, Thailand. Holstein-Frisian cows, lactation 1-8, 30-90 days postpartum with clinical endometritis were enrolled in the study. Conditions during parturition such as dystocia, retained fetal membranes, caesarean section, twins, stillbirth, abortion, etc. were recorded. Clinical endometritis defined as the presence of purulent uterine discharge detectable in vagina 21 days or more postpartum, or mucopurulent discharge detectable in the vagina after 26 days postpartum (Sheldon et al., 2006).

Clinical examination

Vaginal mucus content was evaluated using Metricheck™ device (a stainless steel probe with a semi-spherical rubber cup attached at one end) for sampling the contents of the anterior vagina. Vaginal discharge was scored from 0 to 3 according to Williams et al., 2005: 0 = clear or translucent mucus; 1 = mucus containing flecks of white or off-white pus; 2 = <50 mL exudate containing ≤50% white or off-white mucopurulent material; 3 = >50 mL exudate containing ≥50% purulent material, usually white or yellow, but occasionally sanguineous.

Collection of uterine endometrium discharge

Cows were restrained and cleaned perineum and vulva with saline irrigate solution, disinfectant solution and then dried. The collection of uterine endometrium discharge was operated using technique described previously (Virakul et al., 1995). Briefly, a transcervical guarded swab was used for swabbing cervical discharge. The swab comprised a 0.3 cm diameter cotton wool tip sheathed in a long sterilized stainless steel 45 cm long, 0.6 cm diameter with 1.5x0.5 cm hole at 0.5 cm from the blocked tip and wrapped with plastic breeding sheath with the hole at the same position. To prevent contamination, this guard tube was covered with sanitary sheath before inserted through the vagina, pushed the guard tube pierce through the sanitary sheath when reached external cervical through the cervical canal into the lumen of the uterus, guided by palpation per rectum. In the uterine lumen, the swab was extruded from the guarded tube to swab from the endometrium. Withdrew the swab into the guard tube and removed from the uterus then transferred the swab to the transport media and cultured within 3-4 hours after collection.

Microbiological examination

Bacteriological samples were cultured for aerobic bacteria on sheep blood agar and enriched in brain heart infusion (BHI) medium for 24 h at 37°C. The broths were streaked onto sheep blood agar plates and cultured for a second time 24 h at 37°C. Bacteria were identified on the basis of the characteristics of the colony, Gram stain, morphology, hemolysis, biochemical profile with triple sugar iron (TSI) agar and motility-indole-lysine (MIL) medium, and other standard tests such as citrate, catalase, urease and growth on MacConkey agar. Plates containing 1 or more colony-forming units were regarded as positive bacterial growth. Plates with more than 3 species or with mixed culture of moderate degree or higher were considered contaminated (Werner et al., 2012).

Antimicrobial susceptibility test

The antimicrobial susceptibility was tested by the disc diffusion method and performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines in Mueller-Hinton agar (Malinowski et al., 2010). The following antibacterial agents (Oxoid) were used: amoxicillin with clavulanic acid (AMC; 30 µg), amoxicillin (AML; 10 µg), ampicillin (AMP; 10 µg), cephalexin (CL; 30 µg), cefazolin (KZ; 30 µg), gentamicin (CN; 10 µg), oxytetracycline (OT; 30 µg), streptomycin (S; 10 µg) and sulfamethoxazole-trimethoprim (SXT; 25 µg). Interpretation of the test results were sensitivity (S), intermediate sensitive (I) and resistant (R) were based on CLSI criteria.

Statistical analysis

Prevalence of endometritis was defined as number of cow with abnormal vaginal discharge dived by the number of cow examined (Sheldon et al., 2006). The results of the bacteriological findings and antimicrobial susceptibility were described.

Results

Fifty-six cows from 30 small holder dairy farms were diagnosed as clinical endometritis and had bacterial cultured. The median lactation number was 3 and maximum was 8. The mean for day postpartum was 51.68. Cows with vaginal discharge score 1, 2 and 3 were 2 (3.45%), 48 (82.76%), 8 (13.79%), respectively. Twenty cows were recorded for retained fetal membrane after calving. Bacterial finding was shown in Table 1 and 2, and antimicrobial susceptibility profiles

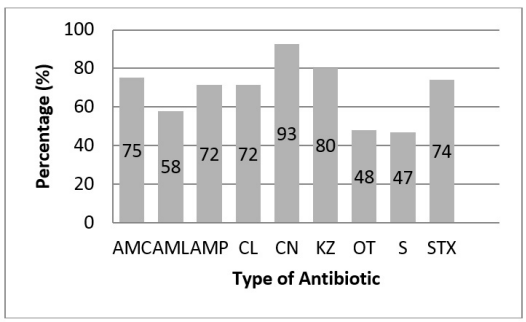

Figure 1. Antimicrobial susceptibility of overall bacterial isolates for 9 antibiotics: AMC = amoxicillin with clavulanic acid, AML = amoxicillin, AMP = ampicillin, CL = cephalexin, CN = gentamicin, KZ = cefazolin, OT = oxytetracycline, S = streptomycin and SXT = sulfamethoxazole-trimethoprim.

Table 1. The bacterial types, number of isolate and percentage of finding.

Gram	Bacterial identification	No. of Isolates	Percentage by group	Percentage by total finding
Positive	*Corynebacterium* spp.	18	31.03	18
	A. pyogenes	14	24.14	14
	Staphylococcus spp.	11	18.97	11
	Streptococcus spp.	9	15.52	9
	Bacillus spp.	5	8.62	5
	Micrococcus spp.	1	1.72	1
Total		58	100	58
Negative	*Escherichia coli*	24	57.14	24
	Pasteurella spp.	6	14.29	6
	Actinobacillus spp.	3	7.14	3
	Enterobacter spp.	3	7.14	3
	Achromobacter spp.	2	4.76	2
	Klebsiella spp.	1	2.38	1
	Proteus spp.	1	2.38	1
	Citrobacter spp.	1	2.38	1
	Erysipelothrix spp.	1	2.38	1
Total		42	100	42
Total findings		100		100

were shown in figure 1, 2 and 3.

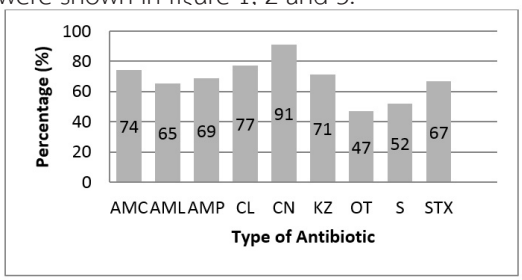

Figure 2. Antimicrobial susceptibility of 58 gram-positive bacterial isolates for 9 antibiotics: AMC = amoxicillin with clavulanic acid, AML = amoxicillin, AMP = ampicillin, CL = cephalexin, CN = gentamicin, KZ = cefazolin, OT = oxytetracycline, S = streptomycin and SXT = sulfamethoxazole-trimethoprim.

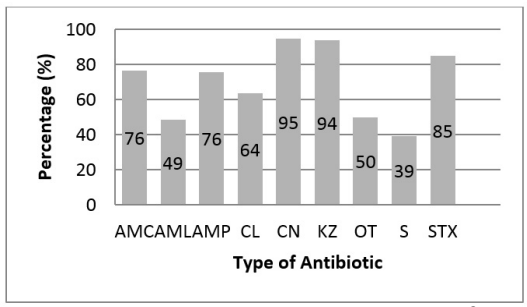

Figure 3. Antimicrobial susceptibility of 42 gram-negative bacterial isolates for 9 antibiotics: AMC = amoxicillin with clavulanic acid, AML = amoxicillin, AMP = ampicillin, CL = cephalexin, CN = gentamicin, KZ = cefazolin, OT = oxytetracycline, S = streptomycin and SXT = sulfamethoxazole-trimethoprim.

Discussion

In the present study, samples with vaginal discharge score 1 had negative results (no growth) in biological examination which related to scoring system to evaluate endometritis. The vaginal mucus is scored for character and odor using the given descriptions. The two scores (2 and 3) are totalled to give the endometritis score (Sheldon and Dobson 2004). Moreover, Sheldon et al., 2006 reported guidelines classify cases with a mucopurulent or purulent vaginal discharge as clinical endometritis only if the cow has calved for more than 21 days. Diagnosis of endometritis based on the presence of pus mixed with the vaginal mucus (LeBlanc et al., 2002; Sheldon and Noakes, 1998; Dohmen et al., 1995). We can assume that pus in the vagina reflects uterine infection but this requires microbiological confirmation (Williams et al., 2005).

Interestingly, 20 of 58 cows in this study had retained fetal membrane after calving. This data supported previous studies that retained fetal membranes the most important risk factors of uterine infection because of increasing bacterial contamination and uterine damaged and providing a favorable environment for bacterial growth (Potter et al., 2010).

In this study, *E. coli* (24%), *Corynebacterium* spp. (18%), *A. pyogenes* (14%), *Staphylococcus* spp. (11%) and *Pasteurella* spp. (6%) were the most frequently isolated. Emphasized on 30-45 days postpartum dairy cows in the study, we found *Corynebacterium* spp. 25.93%, *E. coli* 24.07%, *Bacillus* spp. 20.37%, *Streptococcus* spp. 14.81%, *A. pyogenes* 5.55% and *Staphylococcus* spp. 5.55%. According to previous studies in Thailand, Virakul et al. (1995) investigated in 30 days postpartum dairy cows reported *Staphylococcus aureus* 28.2%, *Corynebacterium pyogene*s 23.1%, Acinetoba*cter calcoaceticus* 10.3% and *E. coli* 17.9%. while Ngarmkum et al., 1993 found *Staphylococcus aureus* 32.14%, *Acinetobacter anitratus* 25.0% *E. coli* 21.43%, *Corynebacterium pyogene*s 14.29%. Our result was a bit different from above reports in the cases of the most bacterial isolated due to different study area and time. Common bacteria isolated from cows with acute metritis and chronic endometritis are *A. pyogenes*, *Prevotella* spp., *Fusobacterium necrophorum* and *Escherichia coli* (Lewis, 1997; Smith et al., 1998; Overton et al., 2003; Sheldon and Dobson, 2004; Sheldon et al., 2006). Other studies also found *Staphylococcus* spp., *Streptococcus* spp. or non-*E.coli* aerobic gram-negative rods (Silva and Lobato, 1998; Kaczmarowski et al., 2004; Jadon et al., 2005). The pathogenic bacteria that are frequently isolated from endometritis cases are *A. pyogenes* and *E. coli*. Because of expense and method's limitation, only aerobic bacteria was investigated. For further study, anaerobic bacteria should be concerned.

From all 9 antimicrobial agents (Figure 2), gentamicin, cephalexin and amoxicillin-clavulanic acid were the 3 most susceptible (91%, 77% and 74%, respectively) of gram-positive aerobic bacteria. While oxytetracycline, streptomycin and sulfamethoxazole-trimethoprim were the 3 most resistant (43%, 25% and 27%, respectively). In figure 3, gram-negative aerobic bacteria was the most susceptible to gentamicin, cefazolin and sulfamethoxazole-trimethoprim

(95%, 94% and 85%, respectively). While oxytetracyclin, amoxicillin, ampicillin and streptomycin were the 4 most resistant (41%, 26%, 24% and 24%, respectively). Gentamicin was the most sensitive antimicrobial agent to overall 100 bacterial isolates (93%), cefazolin (80%) and amoxicillin-clavulanic acid (75%). Less active antimicrobial agents were oxytetracycline (42%) streptomycin (25%) and sulfamethoxazole-trimethoprim (22%). Gentamicin was the most sensitive antibiotics and oxytetracycline was the most resistant for both gram-positive and gram-negative endometritis pathogens. Emphasized on antimicrobial resistant, oxytetracycline was routinely used for uterine infection, especially after calving, intrauterine infusion and/or intramuscular administration. Common antibiotics that could found in dairy farms in this study area were kanamycin, oxytetracycline, penicillin-streptomycin and sulfamethoxazole-trimethoprim. Although oxytetracycline is widely used, it is not the optimum treatment because there is evidence for bacterial resistance to this antimicrobial and high concentration of the antibiotics are required to inhibit bacterial growth when tested in vitro (Sheldon and Dobson, 2004). The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents (Tenover, 2006). The more antibiotics used improperly, the more chances for bacterial mutation.

The devices for sample collection in this study were practical for field research. With artificial insemination technique aseptically, according to no contaminants from microbiological examination, emphasized the advantage of this procedure which was adapted from the previous study (Virakul et al., 1995).

This study provided an update information of aerobic pathogens causing endometritis and their status of antibiotic resistance. Such information is useful for therapeutic treatment planning and antibiotic control usage. In conclusion, the prevalence of endometritis was 45.88%. The most frequently isolated were *E. coli*, *Corynebacterium* spp., and *A. pyogenes*. Gentamicin was the most sensitive antibiotics and oxytetracycline was the most resistant for both gram-positive and gram-negative endometritis pathogens.

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