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Strategies for Diagnosis and Treatment of Uterine Infection in Bovines

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Abstract: Uterine infections are among common diseases that lead to a potential profit reduction in dairy industry due to decreased milk yield and poor fertility. Calving problems, metabolic diseases such as milk fever, ketosis, abomasal displacement and the endocrine milieu affect likelihood of elimination of bacteria could be predisposing factors leading to uterine infections in dairy cows. *Trueperella pyogenes* and *Escherichia coli* are frequently isolated from the bovine uterus and are well known etiological agents of bovine endometritis. Endometritis could be diagnosed by clinical examinations; gloved hand, transrectal uterine palpation and ultrasonography as well as laboratory examinations; cytological, bacteriological and quantitative PCR analyses. The most common method of treatment is either intrauterine or systemic antibiotic administration.Ceftiofur hydrochloride, ceftiofur crystalline-free acid are proven to be the drug of choice for the treatment of postpartum metritis in dairy cows. The advantage of intrauterine infusion of ceftiofur at approximately 6 wk postpartum, between two injections of prostaglandin ($PGF_2\alpha$) two weeks apart, reduced the prevalence of uterine bacterial infection but did not improve the rate of pregnancy in the first 310 days postpartum. Sequential application of estradiol benzoate and oxytocin with intrauterine infusion of rifampicin and systemic administration of oxytetracycline positively affected the clinical cure and uterine involution of buffaloes with toxic puerperal metritis. The infusion of antiseptic agents such as iodine into the postpartum uterus is not recommended. The occurrence of uterine infections can be reduced by minimizing the need for calving assistance through prenatal knowledge of twin birth, calf sex and oversized calves.

Key words: Farm Animals · Uterine Infection · Diagnosis · Antibiotics · Hormones · Managment

INTRODUCTION

Uterine infections are among the most important reproductive disorders causing substantial economic losses in dairy cattle due to decreased milk yield and impaired fertility [1, 2].

During the first 3 weeks after parturition,a diverse quorum of bacteria including *Trueperella pyogenes* (43.5%), *Escherichia coli* (21.5%), *Bacillus spp*. (21.0%) and *Streptococcus uberis* (18.5%) can be frequently isolated from the bovine uterus. Those pathogens are well known as etiological agents of bovine endometritis, but other bacterial species can also be isolated from the bovine uterus post-partum [3-5].

The uterine clearance is a highly dynamic process, during which bacteria show distinct patterns of progression and interactions which lead to the occurrence of clinical endometritis [6]. The efficacy of uterine clearance depends on the immune status of the cow and the load and virulence of the bacteria. Although more than 70% of cows clear the uterine bacteria via innate immune responses, 17- 37% of cows develop clinical endometritis whereas 14- 53% develop subclinical endometritis [7].

Retained placenta, dystocia,twining and stillbirth could be predisposing factors, determining a concomitant delay in uterine involution and expulsion of lochia, disruption of neutrophil function and tissue damage [8, 9]. Establishment of uterine bacterial infection may also depend on metabolic diseases such as milk fever, ketosis and displacement of abomasum, although the specific mechanisms are not clear [10]. Moreover, the endocrine meliu affects the likelihood of elimination of bacteria [9] as bacterial growth is facilitated by progesterone [11, 12] while administration of estrogens or estrous induction enhances elimination of bacterial infection [13].

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In the early postpartum period, infections of the uterus with *E. coli* pave the way for subsequent infection with other bacteria [14], especially *T. pyogenes* [15, 16]. When *T. pyogenes* was isolated from uterine fluids approximately 21 d postpartum, cows developed severe endometritis and were usually infertile at first service. *T. pyogenes* were strongly associated with clinical endometritis when detected at the 34 to 36 d postpartum [17]. It was found that *E. coli* at 34 to 36 d postpartum was not associated with clinical endometritis or reproductive failure [16].

The early diagnosis and treatment of uterine infection is essential for the proper reproductive performance of affected dairy cows. The first postpartum examination is usually performed from 3-5 weeks after parturition to identify animals with a higher risk for reproductive failure [18, 19]. Different diagnostic techniques, including transrectal uterine palpation [20], ultrasonography, vaginoscopic examination [18, 21] culture of uterine fiuids [22] uterine biopsy [23] and uterine cytology [24] contribute to the variation in case defination and treatment options. A vaginoscopic examination to detect abnormal uterine discharge is a more sensitive and specific method for detection of endometritis than transrectal palpation [18, 21]. However, vaginoscopy often fails to identify all cows that are truly at risk of poor reproductive performance.

The most common method of treatment is either intrauterine [18, 25-28] or systemic [29] antibiotic administration. Hormones such as estrogens and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) have been incorporated into treatment protocols for cows suffering from postpartum metritis. The effect of these compounds on uterine motility and defense mechanisms makes them useful treatment alternatives to antimicrobial agents. Estrogen may stimulate uterine tone to evacuate abnormal uterine contents, increase production of mucus that contains host defense compounds and induce estrus, thereby reduce progesterone levels markedly stimulate neutrophil phagocytosis and resistance of the uterus to infection. Much remains to be learned about what initiates and sustains harmful in fiammation of the reproductive tract. Such information is necessary to develop effective treatments for the various forms of disease and more importantly, to develop means to prevent endometritis and cervicitis. In particular, vaccination against specific uterine pathogens and interventions to modulate innate immune response appear to be important avenues for investigation.Presently, the most common recommended management practices for cows in the transition period are likely to be helpful to mitigate the risk of reproductive disease [30]. Management at calving plays an important role in the subsequent reproductive performance of dairy cows. Poor hygiene at parturition or insemination lead to ascending of infectious microorganisms into vagina which can impair uterine involution.Susceptible cows are those which previously suffered from dystocia, retained placenta, twin birth, stillbirth or metabolic disorders [31].

The aim of the present work is to highlight updates for diagnosis and treatment of uterine diseases in cows.

Types of Uterine Infections: The defination of uterine diseases encountered in cattle have been reviewed and articulated according to Sheldon*et al*. [19]:

- *Puerperal metritis* is defined as an animal with an abnormally enlarged uterus and a fetid watery red-brown uterine discharge, associated with signs of systemic illness, decreased milk yield, dullness or other signs of toxemia and fever (>39.5 °C) within 21 days after parturition. Acute puerperal metritis occurs within the first 10–14 days postpartum. It results from contamination of the reproductive tract at parturition and often, but not invariably, follows complicated parturition. Important causative organisms in cattle include *E. coli* and *Trueperella (Arcanobacterium) pyogenes*, but culture-independent studies have demonstrated the dominant role of Gram-negative anaerobic bacteria such as *Prevotellamelaninogenica* and *Fusobacteriumnecrophorum*. The condition is usually acute in onset and milk production is diminished.
- *Clinical metritis* is defined as animal not systemically ill, but has an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days after calving.
- *Clinical endometritis* is characterised by the presence of purulent (>50% pus) uterine discharge detectable in the vagina 21 days or more after parturition, or mucopurulent (Approximately 50% pus, 50% mucus) discharge detectable in the vagina after 26 days.
- *Subclinical endometritis* is defined as a cow with >18% neutrophils in uterine cytology samples collected 21–33 days after calving, or >10% neutrophils at 34–47 days.
- *Pyometra* is defined as the accumulation of purulent material within the uterine lumen in the presence of a persistent corpus luteum and a closed cervix.

Incidence of Uterine Infection: There is abundant evidence that the vast majority of cows have bacterial contamination of the uterus for 2 to 3 weeks after calving [9] with lactational incidence of metritis between 10 and 20%, of clinical endometritis or Purulant vaginal discharge (PVD) approximately 15% and of subclinical or cytological endometritis a further 15% [32].

From the genetic point of view, Bicalho *et al*. [16] found 32 potential virulence genes from 611 E. coli isolates from 374 cows in four herds collected in the first week after calving. They found that six genes; fimH, astA, cdt, kpsMII, ibeA and hlyA were mostly associated with both metritis and clinical endometritis. The fimH adherence gene was present in 87% of uterine *E. coli* isolated from cows and was strongly associated with increased risk of disease. The risk of metritis was highest when this gene was present with one of the other five virulence genes identified.

Diagnosis of Uterine Infection

Clinical Examination

Gloved Hand: At first, cows were inspected for the presence of fresh discharge on the vulva, perineum, or tail. If discharge is not visible externally, cows should be examined vaginally using hygienic precautions. The gloved hand is inserted through the vulva and the mucus contents of the vagina should be withdrawn manually for examination of color and proportion of pus. Endometritisis classified in three categories: clear mucus with flakes of pus (E1), mucopurulent discharge or fluctuating contents in the uterus (E2) and purulent discharge with or without palpable contents in the uterus (E3) [33].

Ultrasonographic Assessment of Uterus and Ovaries: Using rectal linear probe was also performed. Diameter of the uterus, echotexture and thickness of the uterine wall and intraluminal fluid accumulation were evaluated in the cows. Ovarian structures [Follicle and corpus luteum (CL)] should be scanned.The sonographic features of postpartum metritis were in part similar to endometritis, however, there was enormous amount of anechoic fluid in the distended uterus along with echogenic particles. Fissore *et al*. [34] and Melendez *et al*. [35] revealed that diagnosis of pyometra by ultrasonography is done on the basis of high volume of accumulated echogenic uterine content without fetus and cotyledons, closed cervix and corpus luteum on the ovary [19, 34, 36, 37]. Subsequent to therapy, the uterine diameter, fluid accumulation and echogenic particles got reduced.

Transrectal Palpation of the Reproductive Tract: Following inspection, transrectal palpation of the reproductive tract was performed and cervical diameter, location of the uterus, symmetry of the uterine horns, diameter of the (Larger) uterine horn, texture of uterine wall, palpable uterine lumen, dominant palpable ovarian structure including corpus luteum (CL), follicle, or no palpable structures were recorded [9].

Laboratory Examination

Cytological Tests: Smears from endometrial epithelium were collected from uterine horn using a trans-servical guarded swabs [37] and cytological slides were prepared by rolling the cytobrush on a clean glass microscope slide and fixing with cytofixative. The smears were then allowed to dry at room temperature for 30-35 min and stained with a modified Giemsa stain. Cytological assessment determined the percent of neutrophils in order to provide a quantitative assessment of endometrial infiammation [38].

Quantitative PCR(Q-PCR) Analyses: Endometrial epithelium samples were collected from the uterine horn for Q-PCR using a Hauptner biopsy instrument [39]. After collection, samples were immediately fixed in 500ul of RNAfree water and frozen at-80 °C until processing. Total RNA was extracted using RNA extraction kits according to the manufacturing instructions. The concentration and purity of RNA were determined using spectrophotometry. Total RNA was eluted in 30-50 ulRNase free water forcDNA synthesisusing RT-PCR. The relative quantification in the expression of IL1A, IL6, IL17A, TNFα, PGHS2 and ERα genes was estimated as the quotient between Q value of the target gene and a normalization factor (NF) which was calculated based on geometric mean of housekeeping genes Q values [40].

Bacteriological Examination: Malinowski *et al*. [41] determined *in vitro* sensitivity tests of 161 *T. pyogenes* strains and 99 *E. coli* strains isolated from the uteri of 312 cows suffering from metritis/endometritis with clinically diagnosed pathological discharges. Swabs from the lumen of the uterus were aseptically collected and bacteriologically examined according to commonly accepted procedures with the additional use of API tests. Sensitivity to antibiotics was tested by the disk diffusion method and performed in Mueller-Hinton agar. *T. pyogenes* strains were the most sensitive to amoxicillin/clavulanic acid (Amc) (97.3%), bacitracin (96.7%), ceftiofur (95.8%) and cephapirin (77.5%). *E. coli*

strains were the most sensitive to norfloxacin (98.1%), marbofloxacin (95.8%), gentamycin (88%), Amc (80.7%) and ceftiofur (73.1%). *T. pyogenes* and *E. coli* were most resistant to oxytetracycline (63.7 and 31%) respectively. Azawi *et al.* [42] revealed that *E. coli, T. pyogenes*, *Staphylococcus aureus* and *Fusobacterium necrophorum* were the most prevalent bacteria in uterine lumen of buffalo cows suffering from toxic puerperal metritis. Sensitivity test indicated that most of the bacterial isolates were sensitive to rifampicin, oxytetracycline 73.8 and 67.9%, respectively. Sequential application of estradiol benzoate and oxytocin with intrauterine infusion of rifampicin and systemic administration of oxytetracycline positively affected the clinical cure and uterine involution of buffaloes with toxic puerperal metritis.

Treatment of Uterine Infection: A variety of therapeutic methods for clinical endometritis has been reported including systemic [43] or local [25] antibiotics as well as $PGF_2\alpha$ or estradiol administration [18, 43-45]. The results of the antibiotic application for treatment of endometritis are controversial. LeBlanc *et al*. [18] reported that the intrauterine infusion of cephapirin benzathine at 27-33 days in milk (DIM) significantly decreased the time to pregnancy compared to untreated control. On the other hand,the intrauterine infusion of ceftiofur, oxytetracycline or penicillin did not affect the reproductive performance compared to control cows.

Antimicrobial agents may be needed to control infection caused by bacteria and prevent the progression of disease.Compounds from numerous antimicrobial families (e.g., sulfonamides, tetracyclines, ß-lactams, aminoglycosides, cephalosporins) have been used singularly or in combination for treating postpartum metritis. There are extensive arguments concerning the treatment of specific forms of postpartum metritis with antimicrobial agents as the use of antibiotics in dairy cattle could result in a milk-residue violation, a human health risk and /or bacterial resistance.

Antibiotics

Cephalosporins: Acute puerperal metritis responds well to systemic antimicrobial therapy combined, if necessary, with NSAIDs and other supportive measures such as fluid therapy. Cephalosporin antibiotics or penicillin are considered most appropriate for systemic treatment of cows with metritis because they are active against most common pathogens, reach therapeutic levels in endometrial tissues and may help prevent some of the potential sequelae of metritis and endometritis such as endocarditis or renal disease. Systemic administration of ceftiofur hydrochloride improves clinical signs of metritis, but the effects on fertility have not been evaluated [29]. Ceftiofur hydrochloride is recommended as the drug of choice for the treatment of postpartum metritis with dose of 2.2 mg/k g/ BW (2 ml/100 lbs) IM injected for 5 days in dairy cows because it has no withhold for milk and 3 days for meat. The advantage of ceftiofur crystalline-free acid would be the treatment regimen with a recommended dose of 6.6 mg/kg/ BW (1.5 ml/100lbs) given twice 72 h apart with in the middle third or at the base of the ear although ceftiofur crystalline-free acid also has a 13 day withhold for meat but no withhold for milk. Intrauterine infusion of ceftiofur at approximately 6 wk postpartum, between two injections of $PGF_2\alpha$ two weeks apart, reduced the prevalence of uterine bacterial infection with *E. coli* from 10 to 2% and with *A. pyogenes* from 6 to 1% among cows with PVD, but did not improve the probability of pregnancy or the rate of pregnancy in the first 310 d postpartum [25].

Oxytetracycline: Nak *et al.* [46] reported that cows with metritis and are positive for BoHV-4 recovered clinically after the administration of antibiotics and $PGF_2\alpha$. The infected cows are treated with intrauterine administration of oxytetracycline and IM injections of $PGF_2\alpha$ for three consecutive days. Concurrently, with the administration of oxytetracycline and $PGF_2\alpha$, cows with a rectal temperature >39.5°C received an additional treatment with oxytetracycline (i.m) for three consecutive days. According to the antibiotic test result, on day 3 after the last oxytetracycline and $PGF_2\alpha$ administrations, all cows were treated with a combination of amoxicillin and clavulanic acid (i.u.) for three consecutive days. Oxytetracycline requires administration at high levels (11 mg/kg, bid) to maintain uterine tissue concentrations of 5 mcg/g, which is below the minimal inhibitory concentration (MIC) for many strains of pathogenic *T pyogenes*. Drainage of the uterine content may be advantageous but should be attempted only after initiation of antimicrobial therapy and it should be done very carefully because the inflamed uterus may be friable and manipulation of the uterus may result in bacteremia.Liquamycin LA-200 is a long-acting oxytetracycline. Its adoption is still uncertain because of its long withhold time for milk (4 days) and meat (28 days). Intrauterine treatment with 5 g chlortetracycline twice weekly for 2 weeks prevented the negative effects of metritis on fertility and milk yields in multiparous cows [26]. However, this treatment is not approved and would lead to long milk withdrawal.

Cephapirin Benzathine: There is a consistent evidence that the reproductive performance of cows with PVD has improved when treated with a single intrauterine infusion of cephapirin approximately 1 month before first insemination, relative to receiving no treatment [18, 47, 48]. A formulation containing 500 mg of cephapirin benzathine in 19 g emulsifier improved reproductive performance of cows [18]. Treatment with Metricure® was also found to improve fertility in cows with a history of retained fetal membranes, stillbirths, or a vulval discharge after 13 DIM [47].

Hormonal Treatment:

Estradiol Cypionate (Estradiol 17β): It has been used for treating dairy cows affected with metritis or endometritis but it has not been approved for this use [49]. The protocol for the extralabel use of estradiol cypionate is 4 mg IM in cows that have postpartum reproductive problems during the first 10 - 25 days after calving. It was reported that estradiol enhances uterine resistance to infection by increasing uterine motility as well as mucus production and flow, which collectively promote the evacuation of purulent material from the uterus and may enhance uterine involution [50, 51]. Furthermore, it has been reported that the use of estrogens during the postpartum period may be contraindicated. The postpartum use of estrogens has been associated with severe infection of the oviducts and increased incidence of cystic ovarian degeneration [52].

Prostaglandin $F_2\alpha$: Because there is increased concern about bacterial acquisition of antibiotic resistance, $PGF_2\alpha$ would provide an efficacious method of treatment of endometritis. The benefit from $PGF_2\alpha$ administration is believed to arise from induction of estrus in cows having a $PGF_2\alpha$-responsive corpus luteum; physical expulsion of bacterial contaminants and inflammatory products as well as a possible improvement in the uterine defenses under low progesterone [27]. $PGF_2\alpha$ also appears to have pro-inflammatory actions that might enhance neutrophil function [53]. Nonetheless, later studies found no beneficial effect of $PGF_2\alpha$ for treatment of subclinical endometritis [54, 55], therefore, the combined literature suggests that $PGF_2\alpha$ is not an efficacious method to treat subclinical endometritis.

Treatment with Antiseptic Agents: Antiseptic agents such as iodine, chlorhexidine and saline have been infused into the uterus, but there have been few studies to determine the efficacy of these compounds on postpartum metritis. The irritating nature of such solutions is thought to increase uterine tone, blood flow and defense mechanisms [22]. The induced inflammatory response of the uterus is thought to reduce the bacteria level within the uterus and aid in evacuating abnormal uterine fluid. Cattle infused with an irritating chemical reportedly had a shortened estrous cycle (8-10 days) when the solution was administered early in the diestrous period. In general, the infusion of antiseptic substances into the postpartum uterus is not recommended. This method of treatment can lead to iatrogenic mechanical trauma to the genital tract and secondary bacterial infection through iatrogenic contamination of the genital tract.

Immune Modulators: Rahim *et al*. (56) evaluated the efficacy of treatment of postpartum endometritis in cows with intrauterine infusion with hyperimmune serum (HS). They found higher cure rate and reproductive performance in cows treated withhyperimmune (64.3%), Oxytertracyline (OTC, 61.5%) compared to prostaglandin treated cows (PG, 72.3%). Conception rate to all services for cows with endometritis was 52.9% in HS group, 57.1% in OTC and in PG 62.1% compared to 66.7% in HC. The difference in the conception rate between the HS and HC group was not significant. HS could not be the antibiotic alternative treatment choice for postpartum endometritis in dairy cattle.

Management Practices: The occurrence of uterine infections can be reduced by minimizing the need for assistance at calving through prenatal knowledge of twin birth, calfsex and oversized calves. Calving assistance should only be carried out when necessary and appropriate hygiene should be used to decrease the incidence of uterine infections [57].

CONCLUSION

It was concluded from this work that ceftiofurhydrochloride and ceftiofur crystalline-free acid are proven to be the drug of choice for the treatment of postpartum metritis in dairy cows. Injections of $PGF_2\alpha$ or sequential application of estradiol benzoate and oxytocin positively affected the clinical cure and uterine involution in cows with toxic puerperal metritis. Uterine infection can be minimized through necessary calving assistance, appropriate hygiene and prenatal knowledge of twin birth, calf sex and oversized calves.

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