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COMPARATIVE CLINICAL STUDY OF DIFFERENT TREATMENT ROUTES OF TOXIC PUERPERAL METRITIS IN DAIRY COWS IN IRAQ.

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ABSTRACT

The objective of the present study was to evaluate the effect of the route of treatment on future fertility in dairy cows with toxic puerperal metritis. This study was carried out on a dairy farm in Al-Latifya south of Baghdad that comprises Holestien, Friesian crossbreeds. A total number of 46 dairy cows during early postpartum period infected with toxic puerperal metritis were selected out the rest of the herd that start calving from the beginning of November 2008 till the end of October the year after. According to the routes of treatment, they were allotted into four groups (10 cows) each. Systemic administration of oxytetracycline antibiotic gave the best result in this study when it was assessed by the reproductive performance achieved compared with other routes of treatment of the same antibiotic. Meanwhile high percentage of toxic puerperal metritis was recorded in cows suffering from dystocia and retained fetal membranes (69.5, 73.91% respectively). The result recorded that high percentage of dairy cows with toxic puerperal metritis were in primiparous (First calving) cows. According to the months of the year it was found that increased percentage of toxic puerperal metritis was recorded in cows calved during January and February (42.3, 31.8% respectively). Bacterial culture results showed that *E.coil, Klebsiella spp staphylococcus spp, streptococcus spp,* and *proteus spp.* were the common bacteria isolated from uteri of cows that suffered from toxic puerperal metritis.

INTRODUCTION

Toxic puerperal metritis ranks among the top health problems of fresh cows , it is an inflammation of the muscular wall of the uterus and endometrium (40, 30, 31). Dairy Cows with toxic Puerperal metritis usually die within 2-10 days or recover (20). Cows recovered from toxic puerperal metritis usually develop metritis or endometritis and both conditions can become chronic (24). Causing high economic Losses due to prolonged days open and inter calving interval resulting in involuntary culling (13, 14). Affected animals had fever with fetid reddish brown uterine discharge and varying degrees of depression, reduced appetite, dehydration and decreased milk production (33, 31). Dystocia and retained fetal membranes were the most significant predisposing factors for toxic puerperal metritis, they provide an excellent substrate for bacterial growth (40). Incidence of postpartum toxic metritis in cows with retention of fetal membranes can be as high as 90% (25), also excessive stretching of the uterus such as twins, improper calving assistance can decrease uterine contractility (17), the contractions of the myometrium and secretions from the endometrial glands also help to remove potentially harmful bacteria (32), the result of decrease contractility leads to retention of fluids and membranes beyond the normal periods providing excellent media for bacterial growth and it reduces the ability of uterine white blood cells to remove bacteria (25).

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Therapy used for uterine infection should eliminate pathogenes from the uterus and its success depends on evacuation of uterine fluids, sensitivity of the causative bacteria to the antibiotic used, and its concentration, the frequency used in treatment and the degree of exposure of the endometrium to the drug used, (3, 19, 29). Oxytetracycline is abroad spectrum antibiotic indicated for the treatment and control of infections caused by bacteria sensitive to this antibiotic (36), its antibacterial efficacy against many infections caused by gram (+) and gram (-) bacteria are well–documented (18), it can be used for intra uterine therapy (35, 18, 7, 10, 36), which was also indicated in the treatment and prophylaxis of postpartum endometritis in the cow (37, 22). Systemic therapy offers many advantages, the moment of withdrawal is generally well established, possible distribution for the entire wall of the uterus and it seems to be less harmful to the uterine environment (39). Several studies recommended the systemic use of antibiotics in cows with toxic puerperal metritis (7, 19, 1, 36). This study was planned and carried out to evaluate and compare the response of toxic of puerperal metritis among different treatments routes with oxytetracycline antibiotic in dairy cows.

MATERIALS AND METHODS

ANIMALS

The current study was conducted on an imported dairy herd that comprises of Holstein, Friesian cross breeds. Cows used in this study were 46 dairy cows infected with toxic puerperal metritis from which 40 cows were selected for the purpose of this study according to the sensitivity of the causative agent to oxytetracycline.

CLINICAL EXAMINATION

General examination comprised monitoring clinical signs such as depression, reduced appetite, dehydration, increase respiratory rate, arched back, colic, pain, presence of fresh discharge on the vulva, perineum or tail. Rectal palpation of reproductive tract was performed on each cow included in this study. The case history, clinical signs and clinical examinations were all used to diagnose the case of toxic puerperal metritis together with two important criteria:-

1- Elevated body temperature.

2-Uterus was located in the abdomen with fetid reddish brown discharge, and abscence of longitudinal rauge.

UTERINE SWAB COLLECTION AND BACTERIOLOGY

Sampling for bacteriological examination was preformed immediately after diagnosis of toxic puerperal metritis. Swabs were collected through the vulva into the vagina after thorough cleaning and disinfection using vagino scope and a catheter containing the cotton swab was introduced near the cervical opening then a metal silk was pushed from the outer opening of the catheter to introduce the cotton swab inside the cervical opening and after rotation of the swab 3-5 times it was then pulled out and were transferred into sterile tubes containing nutrient broth as transport media then transported to the laboratory at $4c^0$, some samples were collected directly from the discharged fluids and immediately processed for bacteriological examination. Swabs were cultured aerobically on sheep blood agar, MacConkey agar, nutrient agar, staphylococcus medium 110 and heated blood agar and anaerobically by candle jar and CO_2 10% together with gas pak. Cultures were examined after 24-48 hours

incubation at 37c^0 for aerobic growth. Identification of bacteria was based on the characteristics of colony, heamolysis, gram stain, morphology catalase test, coagulase test, oxidase test and indole production test.

STUDY DESIGN

Cows with toxic puerperal metritis that were sensitive to oxytetracycline antibiotic alotted into 4 equal groups (10 cows each):

Group1:-Treated systemically by intramuscular injection I/M of oxytetracycline (ALamycin 10% (Nor brook Laboratories GB Limited)) antibiotic in a dose of 4 mg/kg body weight for 3 successive days.

Group2:-Treated locally by intrauterine infusion I/U after manual evacuation of uterine fluid by oxytetracycline antibiotic in a dose of 2 gm diluted with 50 ml distilled water for 3 successive days.

Group3:-Treated systemically and locally at the same time with the same dose of groups (1, 2) for 3 successive days.

Group4:-Evacuation of uterine fluids by massage only until complete evacuation.

Follow- up examination:

- Each animal was re-examined 10 days after diagnosis and treatment.

- Animals without any pathological discharge (pus) considered clinically cured.

- The uterine involution was assessed by gynecological examination at 10,20,40 days intervals according to the following procedures:

1- Monitoring and recording the general health status and the character of fluid present during vaginal examination.

2- Completion of uterine involution through transrectal palpation of the uterus, cervix and ovaries, this was assessed on the following basis:

- Reduction in the size of uterine body and horns to almost the uniform size

- Normal location (more forward in the pelvic cavity).

- Normal tonus and consistency.

- Absence of pathological discharge.

RESULTS AND DISCUSSION

Cows were observed 3 times daily for at least 1/2 hour each time by experienced farm workers. Any cow that stood firmly to be mounted by other cows and show a clear, glassy mucous discharge from the vulva considered in estrus and confirmed by transrectal palpation.

STATISTICAL ANALYSIS

(F-test) was used in the analysis of first estrus after calving, first insemination after calving, average number of inseminations per conception and days open. The percent rate was used for normal calving, dystocia, rate of retained fetal membranes, rate of infection in different months of the year and pregnancy rate from first insemination, mentioned by (27).

The results of the present study revealed high percentage of toxic puerperal metritis cases were due to dystocia and retained fetal membranes 69.5% and 73.9% respectively (Table 1), with high percentage of infection was recorded in newly calved heifers (primiparous cows) 43.4% and low percentage 5% was recorded in multiparous cows, the fourth and fifth calving (Figure.1).

According to the calving, throughout months of the year it was found that calvings in January and February were associated with high percentage of infection with toxic puerperal metritis 42.3 and 31.8% respectively (Figure 2).

Table 1: Clinical observations on type of calving and retention of fetal membranes of study cows

Observations	Total No.	No of infeded cows T.P.M* %
Normal calvings	46	14 / 30.4
Abnormal calvings (dystocia)	46	32 / 69.5
Non-RFM**	46	12 / 26.08
RFM**	46	34 / 73.9

***Toxic puerperal metritis.**
**** Retained fetal membranes.**

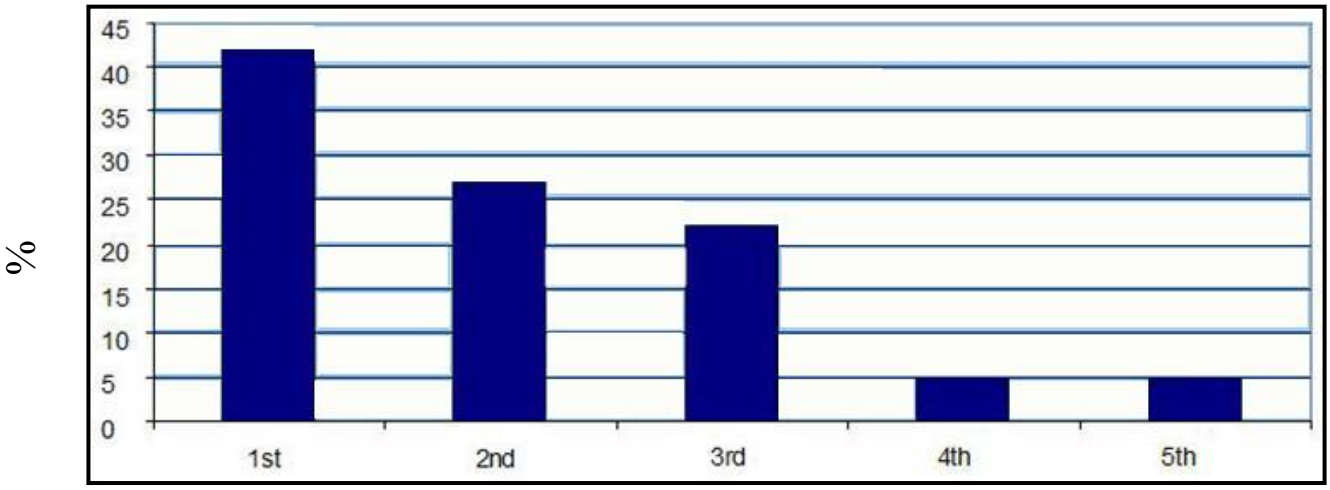

Figure1: Relationship between Number of calvings and toxic puerperal metritis percentage of study cows

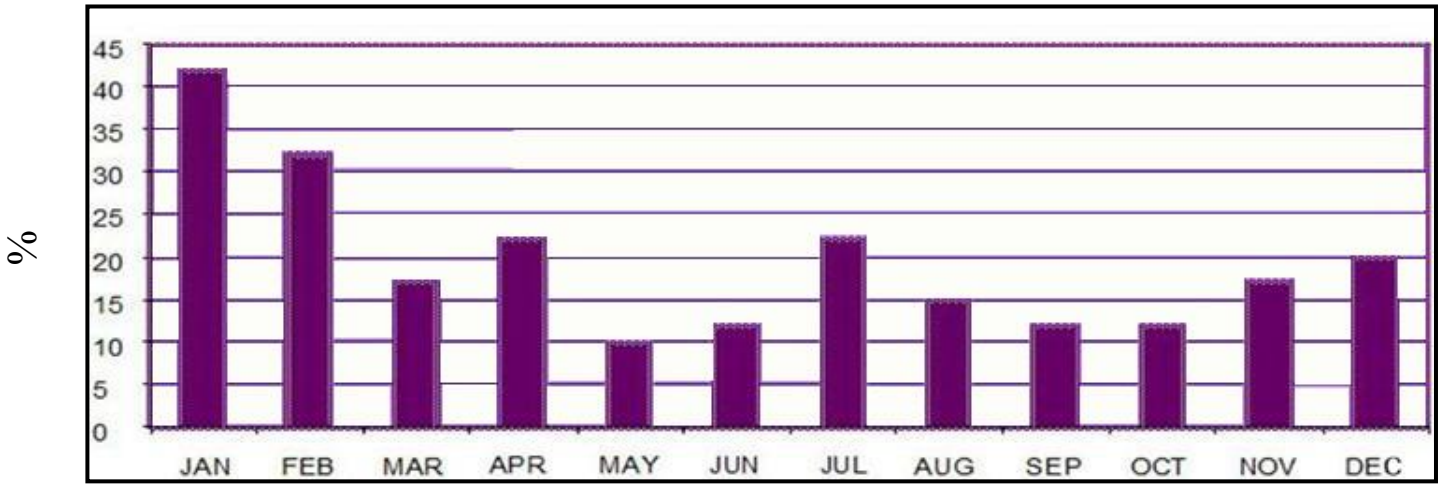

Figure2: Relationship between calvings in months of the year and percentage of toxic puerperal metritis of study cows

The results of bacterial isolation of collected swabs in this study showed the presence of high rate of *Escherichia coli* 37.5%, while the rate of isolation of *Klebsiella spp, Staphylococcus spp, Streptococcus* and *Proteus spp* were 20, 20, 17.5 and 5% respectively (Table 2).

Table 2: Type of bacteria isolated from cows infected with toxic puerperal metritis

Isolated bacteria	No. of swabs	No. of (+) swabs	%
E. Coli	40	15	37.5
Klebsiella spp	40	8	20
Staphylococcus spp	40	8	20
Streptococcus spp	40	7	17.5
Proteus spp	40	2	5

In relation to estrus manifestation, cows with toxic puerperal metritis of the first and fourth treatment groups showed the shorter periods for the manifestation of first postpartum estrus 23 and 27.6 days respectively , while cows of the second and third treatment groups were late to manifest first postpartum estrus 50.9 and 57.2 days respectively with ($p<0.05$) significancy (Table 3).

In regard of first insemination , shorter periods were recorded for the cows of the first and second treatment groups 65.3 and 68.3 respectively meanwhile the cows of the fourth and third groups needed longer periods 78.2 and 100.2 days respectively to be firstly inseminated with significancy of ($p<0.05$) (Table 3).

The results indicated that the first treatment group showed the shortest period of days open 133.9 days shorter than second and third groups 159.6 and 156.9 respectively while the fourth group recorded the longest period of days open 249 days with a significancy of ($p< 0.05$). (Table3).

The results revealed that number of inseminations perconception were low in the cows of the first , second and third treatment groups 1.7, 1.8 and 1.9 insemination respectively while in the fourth group it was high 3.1 inseminations perconception with a significancy of ($p< 0.05$) (Table 3).

Table 3:Reproductive performance for cows of different treatment groups

Groups	First postcalving Estrus(day)	First Insemination (day)	No.of (Insem.) Insemination Perconception	Days Open (Day)	NO.of Preg. Cows	No.of Non Preg.cow	% Of pregnancy
First	a 23 4.07±	a 65.3 4.59±	a 1.7	a 133.9 8.87±	10	0	%100
Second	b 50.9 6.83±	a 68.3 5.97±	a 1.8	a 159.6 19.60±	10	0	%100
Third	b 57.2 9.98±	b 100.2 9.37±	a 1.9	a 156.9 23.38±	10	0	%100
Fourth	a 27.6 2.56±	a 78.2 9.12±	b 3.1	b 249 33.48±	10	0	%100

(means±SE)

Means with different superscripts within columns differ significantly ($p<0.05$).

The results indicated that pregnancy rate from the first insemination was higher in the cows of the first treatment group 40% than those in the second and third group 30% while in the fourth group it was 0% (Table 4).

Table 4: Pregnancy rate from first insemination in cows of different treatment groups

	Treatment groups			
	First group	Second group	Third group	fourth group
Pregnancy rate from first insemination	40%	30%	30%	0%

Result of the study indicated high incidence of toxic puerperal metritis was associated with difficult calving (dystocia) and retained fetal membranes (69.5 and 73.9% respectively), this leads to diminished ability of the uterus to eliminate contaminated organisms providing an excellent substrate for bacterial growth (40), also excessive stretching of the uterus in such conditions, causes decreased uterine contractility (17) which in turn lead to retention of fluids and membranes beyond the normal period providing excellent media for bacterial growth with reduced ability of uterine white blood cells to get rid of bacteria (25). In the present study high percentage of infected cows were primiparous (first calvings), it may be due to the lack of the role of humoral immunity in the defense and cleaning of the uterus postpartum, the heifers may not have adequate levels of antibodies (32, 3) also it may be related to the unsuitable selection of heifers for breeding according to the preferable age, size and weight, leading to increase chances of dystocia which result in high risk of contamination (23, 17), mean while low rate of infection with toxic puerperal metritis associated with the multiparous cows of the fourth and fifth calvings, they had an increased level of antibodies against common pathogens that were responsible for causing the infection (9). According to the months of the year, toxic puerperal metritis cases were high in cows calved during January and February, this can be attributed to large number of heifer calvings in the forementioned months associated with difficult calving (dystocia) that result in high rate of contamination and infection. Results of the bacteriological examination in the present study suggested that uterine infection may occurred as a result of ascending post partum contamination by non-specific environmental organisms , that may explain the high rates of bacterial isolates from the collected samples (28, 2, 21, 20). The infection of the uterus with *E.Coli* appear to pave the way for subsequent infection with other bacteria (42, 8). Also the presence of *E. Coli*, *Proteus spp* as (Gram-) and *Streptococcus spp* as (Gram +) which was considered as inhabitant of the digestive tract that start to multiply during their invasion to the uterus in complicated parturitions (4, 6). The result of the current study recorded shorter period of first postpartum estrus manifestation of the cows in the first treatment group, this finding can be explained mainly due to the great response of systemic treatment with oxytetracycline antibiotic after performing the sensitivity test for all animals included in this study. In cows treated systemically possible distribution of the antibiotic include the entire wall of the uterus in addition it had less effect on the uterine defense mechanism (39). Removal of the inflammatory fluids resulting in rapid uterine involution and cycling of the cow (38). Meanwhile for the cows in

the fourth treatment group the presence of bacterial toxins stimulates the uterus to secret prostaglandin $F2_x$ that hasten the uterine involution leading to normal cycling of the cows (32, 12). In cows of the second and third treatment groups with intrauterine infusion there may be no certainty that the drug is distributed through all layers of the uterus,and the deep layers of the uterine wall receive minimum levels of antibiotic in the best case (30, 36). Results of the present study indicated that cows in the first, second treatment groups had shorter period to be firstly inseminated, this was maybe because they needed less time to recover, regaining their healthy status and consequently resuming their estrus shortly with their utri free off any inflammatory discharge ,then they were inseminated earlier than the cows in the third and fourth treatment groups that may be recovered from toxic puerperal metritis but they develop metritis or endometritis (41, 24) that needed to be retreated during estrus instead of insemination. The length of days open in cows of the first treatment group was shorter than the cows of the second and third treatment groups, this can be attributed to treatment with antibiotics systemically (39). While the second, third and fourth groups had longer days open because they may recovered from toxic puerperal metritis ,then they may develop sub clinical endomertitis after the clinical signs had resolved, animals with sub clinical disease take longer time to conceive and had conception rates about half those of normal animals (15). Results of this study recorded less number of inseminations per conceptions in the first, second and third treatment groups this might be due to that they were efficiently treated for toxic puerperal metritis or metritis (26, 5). While in the fourth group higher rate was recorded as a result of shifting of the infection to the chronic or sub clinical type which needed more number of insemination per conception (15). Also sometimes toxic puerperal metritis that resulted from retained fetal membranes may inhibit the uterine defense mechanism which acts as an obstacle for spontaneous recovery of the uterus (32,11). The pregnancy from the first insemination in the cows of the first group was higher than those of the second and third groups this might be due to the rapid response of the cows of the first group to systemic treatment that hasten regaining their fertility (35). In addition no further complication occurred to cause sub clinical or chronic infection that need higher rate of inseminations per conception (15). While in the fourth group there was no rapid response to the method of treatment which resulted in complications lead to sub clinical or chronic infections (15) . In conclusion systemic administration rout of oxytetracycline to treat cows with toxic puerperal metritis gave the best results in comparison with other administration routes.

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دراسة سريرية مقارنة لمختلف أساليب المعالجة لالتهاب الرحم الانتاني في أبقار الحليب في العراق

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الملخص

الغاية من الدراسة الحالية هي تقويم أسلوب العلاج لالتهاب الرحم الانتاني في أبقار الحليب وتأثير ذلك في مستقبل الخصوبة لهذه الأبقار. أجريت هذه الدراسة في حقل يضم ابقار من سلالتي الهولشتاين والفريزيان في منطقة اللطيفية جنوب بغداد. وقد كان العدد الكلي للأبقار في الدراسة هو **46** بقرة مصابة بالتهاب الرحم الانتاني, تم اختيارها من ولادات القطيع التي بدأت في عام **2008** منذ بداية شهر تشرين الثاني حتى نهاية شهر تشرين اول في السنة اللاحقة, وعلى أساس أسلوب المعالجة تم تقسيم أبقار التجربة الى **4** مجاميع تتكون كل منها من **10** ابقار. وقد بينت نتائج الدراسة الحالية الى ان أسلوب العلاج الجهازي بالمضاد الحيوي الاوكسي تتراسايكلين قد أعطى أفضل النتائج اعتماداً على مستوى الأداء التناسلي المتحقق مقارنة بأساليب المعالجة الأخرى للمضاد الحيوي نفسه.

وقد كانت اعلى نسبة إصابة بالتهاب الرحم ألانتاني هي في الأبقار التي عانت من عسر الولادة وبقايا الاغشية الجنينية (**69.5** و**73.91**%) على التوالي. كما ان نسبة الإصابة في الأبقار ذات الولادة الواحدة هي اعلى منها في الأبقار متعددة الولادات.

وكانت أعلى نسبة للإصابة بالمرض في شهري كانون الثاني وشباط (**42,3** و**31,8**%) على التوالي. اما أكثر انواع البكتيريا المعزولة شيوعاً من الرحم لاابقار الدراسة فهي الاشيرشيا القولونية, الكلبيسيلا, المكورات العنقودية, المكورات السبحية والمتقلبات.

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