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Treatment of toxic puerperal metritis in Iraqi buffalo cows

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ABSTRACT

The objective of the present study was to evaluate the efficiency of using antibiotics with some hormonal preparations for the treatment of toxic puerperal metritis in buffalo cows. Data were collected from 42 buffaloes with toxic puerperal metritis in two large buffalo herds. Swabs for bacteriology and biopsies for histopathology were collected from uterine lumen from each buffalo included in the present study. Results revealed that the most prevalent bacteria in uterine lumen were *E. coli, Archanobacterium pyogenes, Staphylococcus aureus* and *Fusobacterium necrophorum* in buffalo cows with toxic puerperal metritis. Sensitivity test indicated that most of the bacterial isolates were sensitive to rifampicin and oxytetracycline 73.8% and 67.9%, respectively. Treatment of buffaloes with rifampicin and oxytetracycline showed a high level of clinical cure and uterine involution. In conclusion, 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.

Key words: buffalo cow, toxic puerperal metritis, bacteriology, oxytetracycline, rifampicin

Introduction

Toxic puerperal metritis is one of the most important diseases in buffaloes with a high mortality rate postpartum (JAINUDEEN, 1986). Dairy cows with toxic puerperal metritis usually die within 2-10 days or recover (LEWIS, 1997). Cows recovered from toxic puerperal metritis usually develop metritis or endometritis and both conditions can become chronic (MELENDEZ et al., 2004), causing high economic losses due to prolonged open days and prolonged intercalving interval, resulting in involuntary culling

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(JAINUDEEN, 1986; KARIMI et al., 2004). Iraqi buffaloes were considered with low reproductive performance and increased average length of calving interval (AZAWI and TAHA, 2002). Studies concerning morbidity and mortality rate of buffalo cows affected with toxic puerperal metritis have not been published.

Therapy used for uterine infection should eliminate pathogens from the uterus, and should result in the shortest possible withdrawal periods for milk and meat. Success in the treatment of uterine infections depends on evacuation of the uterine fluids, susceptibility of the infectious agents to the used drug, concentration and frequency of drug use and the exposure of the entire endometrium to the drug (ROBERTS, 1986; BONDURANT, 1999; LEBLANC et al., 2002; NOAKES et al., 2002). Evacuation of the uterus contributes to the success of further antibiotic therapy. Evacuation can be done by hormones to expel the fluid or hasten the onset of estrus (LEWIS, 1997). When fluids are expelled, the effectiveness of antibiotics in clearing the remaining infection is improved. The antibiotic should be active against the main uterine pathogens and should maintain its activity in the environment of the uterus. Oxytetracycline is a broad spectrum antibiotic and is indicated for the treatment and control of infections caused by or associated with oxytetracycline sensitive, rapidly growing bacteria (SHELDON et al., 2004b). Its antibacterial efficacy against many infections caused by gram-positive and gram-negative bacteria are well-documented (BRETZLAFF, 1986; KONIGSSON et al., 2001). This antibiotic may also be used by the intrauterine route (SEGUIN et al., 1974; OXENDER and SEGUIN, 1976; BRETZLAFF, 1986; SHELDON and NOAKES, 1998; KONIGSSON et al., 2001; DRILLICH et al., 2001; HAJURK et al., 2003; SHELDON et al., 2004b). Intrauterine administration represents a useful therapy, especially in the treatment and prophylaxis of postpartum endometritis in the cow (CHAUDHERY et al., 1987; KHAN and KHAN, 1989; PATERIA et al., 1992; SINGH et al., 2000; MALINOWSK et al., 2004). Systemic treatment is best if antibiotics are subjected to degradation by conditions in the uterine lumen. An animal affected with toxic puerperal metritis has an invasion of microorganisms into deeper layers of the uterine wall. This would imply that effective concentrations of the antibiotic would be desired in all regions of the genital tract. Recent studies recommended the systemic use of antibiotics in cows with toxic puerperal metritis (DRILLICH et al., 2001; LEBLANC et al., 2002; AMIRIDIS et al., 2003; SHELDON et al., 2004a). The present study was designed to evaluate the treatment of toxic puerperal metritis using rifampicin as the intrauterine route, which has not been used either in cattle or in buffaloes, and oxytetracycline as a systemic route of administration with hormonal preparations as estradiol and oxytocin for evacuation of discharge from the uterus.

Materials and methods

Animals. The present study was conducted on lactating Iraqi northern buffalo cows in two large herds in the Nineveh province. Buffalo cows included in this study had calved recently (4-10 days) and aged 5 to 15 years. All buffalo cows included in the present study were the local Iraqi northern breed. These two private buffalo dairy herds consisted of approximately 180 and 250 buffaloes. There were 42 buffalo cows with toxic puerperal metritis(in both herds) while a control group consisted of 6 buffalo cows that calved normally at least 10 days before and expelled their fetal membranes within 12 h postpartum with no any abnormal clinical signs mentioned later.

Clinical examinations. The general health examination of other clinical signs such as arched back, colic, pain, presence of fresh discharge on the vulva, perineum, or tail, rectal examination of uterus, uterine tubes, ovaries (if palpable), and cervix was performed on each buffalo cow included in this study. Depending on case history, clinical signs, and clinical examinations, the buffalo cows referred as toxic puerperal metritis required to meet the following two criteria that were used to diagnose toxic puerperal metritis: 1) buffalo cows must have been febrile (i.e. rectal temperature >39 ºC), recently calved and 2) must have had a flaccid, non- retractable uterus that was located in the abdomen, and a cervical diameter >7.5 cm and a watery, fetid vulval discharge.

Uterine swab collection and bacteriology. Sampling for bacteriological examination was performed immediately after diagnosis of toxic puerperal metritis. Swabs were transferred into sterile tubes containing thioglycolate broth as a transport media and transported to the laboratory at 4 °C and immediately processed for bacteriological examination. Swabs were cultured aerobically on sheep blood agar, MacConkey agar and nutrient agar and anaerobically on sheep blood agar. Cultures were examined after 24 h incubation at 37 °C for aerobic growth and 7 days for the anaerobic bacteria. Identification of bacteria was based on the characteristics of colony, hemolysis, gram stain, morphology, catalase test, coagulase test, oxidase test, indole production test, methyl red test, Voges-Proskauer test, citrate production test and sugar utilization, and confirmed by API system (API 20E, API 20A, API Coryn, API Staph, API Strep) supplied by (bioMerieux SA, 69280 Marcy L'Etoile, France). Some isolates were re-confirmed by sending these isolates to the Central Health Laboratory, Ministry of Health, Baghdad.

Biopsy collection and histopathology. Biopsies were taken following the culturing procedure. Separate tissue samples were obtained from each uterine horn with the biopsy instrument. The endometrial biopsy was immediately placed into a bottle containing 10% formal saline solution and stored at 4°C until preparation for sectioning, which included dehydration, clearing, embedding, sectioning, and staining, performed according to the methods described by LUNA (1968).

Estimation of polymorphonuclear cells. A fluid aliquot was collected from each buffalo cow included in this study, aspirated from the uterine lumen using sterile uterine catheter and transferred into sterile tubes, then transported to the laboratory at 4°C for the determination of the percentage of polymorphonuclear cells (KASIMANICKAM et al., 2005).

Method of treatments. Buffalo cows with toxic puerperal metritis were subjected to the treatment regimen using rifampicin 1.5 g as intrauterine route, oxytetracycline 10mg/kg B. W. as intramuscular route every 48 h three times and a hormonal preparations using estradiol benzoate 5 mg intramuscularly as a single dose followed by oxytocin 100 IU after 12 h of estradiol administration. There was no control group in buffaloes affected with toxic puerperal metritis because of threat to life.

Follow-up examination. Each animal was re-examined 14 days after diagnosis and treatment. Clinical cure was initially defined as the absence of any pus discharge at the follow-up examination, as well as, the estimation of polymorphonuclear cells of treated buffalo cows. Animals that were not clinically cured received a second dose of the same treatment as initially administered. These animals were then examined for a third and final time after an additional 14 days. Any animal that still had pus discharge at the third examination was recorded as a clinical failure. The uterine involution was assessed by gynecological examinations at 14, 28, and 42 days intervals, according to the following procedures: 1) recording the general health status and character of fluid determined during vaginal examination, 2) palpation of the uterus, cervix and ovaries (if palpable). The completion of uterine involution was determined retrospectively on the basis of the following: 1-Stabilization of uterine dimensions (reduction in size of uterine body and horns to a more uniform size). 2-Location in the body cavity (more forward in the pelvic cavity). 3-Normal tonus and consistency (large, soft, pliable and more turgid). 4-Absence of pathological cervical discharge. The uterine involution was considered complete when the uterine horns were found to be almost symmetrical and the entire uterus was found positioned in or at the edge of the pelvic cavity.

Statistical analysis. Statistical analyses were performed with the software SIGMA SAT, Jandel scientific software V2.0, 2004. The differences in PMNs were tested by variance analysis (ANOVA) and least significance differences (LSD). Student two-sample *t*-test was performed for the difference between two means of rectal temperature, pulse rate, and respiration rate.

Results

The clinical examination of all animals suffering from toxic puerperal metritis indicated: anorexia, depression, staggering gait, paresis, difficulty in rising, constipation with black scanty feces, frequent and recurrent painful straining, high rectal temperature

Table 1. Prevalence of bacterial isolates from the uterus of buffalo cows with toxic puerperal metritis

	Toxic puerperal metritis		Control group	
	Uterus		Uterus	
Bacterial isolates	No	%	No	%
Archanobacterium pyogenes	18	16.67	1	7.14
Bacillus licheniformis	4	3.70	2	14.28
Prevotella melaninogenica	6	5.55	-	-
Citrobacter braaki	2	1.85	-	-
Citrobacter freundii	2	1.85	-	-
Clostridium tetani	6	5.55	-	-
E. coli	20	18.52	4	28.57
Fusobacterium necrophorum	10	9.26	-	-
Klebsiella pneumoniae	3	2.78	-	-
Lactobacillus fermentus	-	-	1	7.14
Peptococcus niger	4	3.70	-	-
Proteus mirabilis	2	1.85	2	14.28
Pseudomonas aeruginosa	2	1.85	2	14.28
Staphylococcus aureus	14	12.96	2	14.28
Staphylococcus epidermidis	5	4.63	-	-
Streptococcus intermedius	1	0.92	-	-
Streptococcus pyogenes	4	3.70	-	-
Streptococcus uberis	5	4.63	-	-
Total	108	100	14	100

Table 2. Mean ± SE of polymorphonuclear cells (PMN) and character of discharge in eatment group of buffalo cows affected with toxic puerperal metritis and the control group with normal puerperium

Groups	% PMN before treatment	% PMN 14 days post-treatment	% PMN 28 days post-treatment	Character of discharge 28 days post-treatment					
				No discharge		Purulent		Clear discharge	
				N°	%	N°	%	N°	%
Toxic puerperal metritis group (n=42)	63.3 ± 4.18[ac]	48.4 ± 3.6[a]	34.9 ± 2.95[ad]	---	----	16	38.1	26	* 61.9
Control group (Normal puerperium)	47.63 ± 6.1[b]	33.85 ± 5.6[b]	23.78 ± 4.1[b]	---	-----	-----	-----	6	100

Superscripts a, b (P<0.05); c, d (P<0.01).

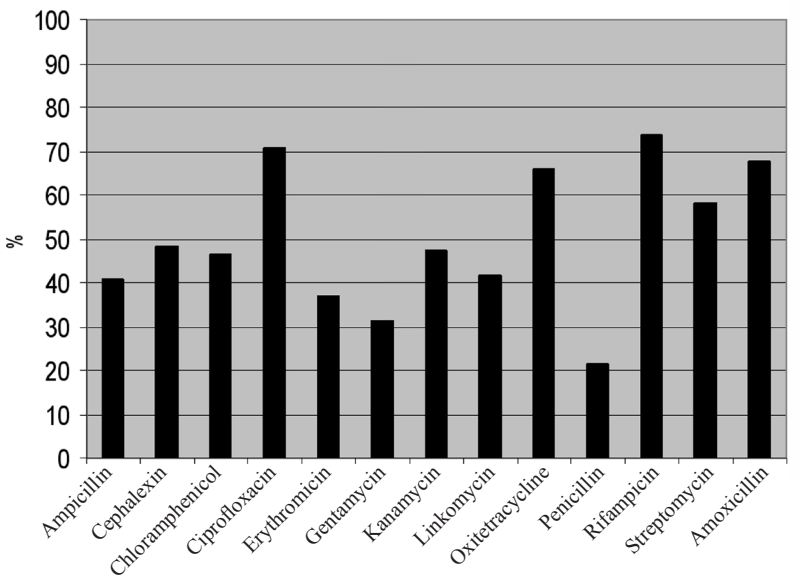

Fig.1. Results of sensitivity tests of various antibiotics on bacterial isolated from the uterus of buffalo cows.

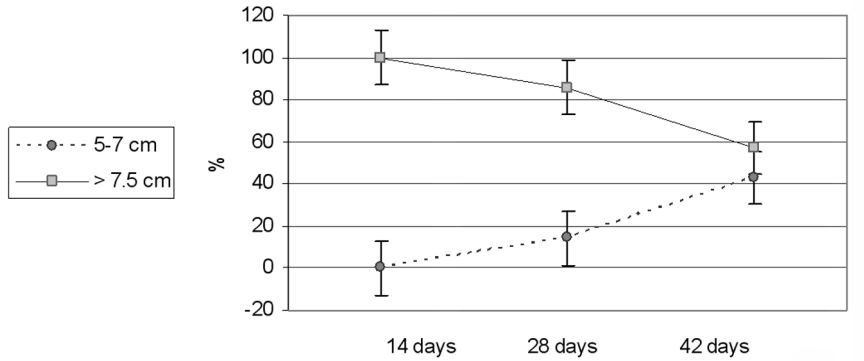

Fig. 2. Cervical diameter of buffaloes at 14, 28, and 42 days post-treatment

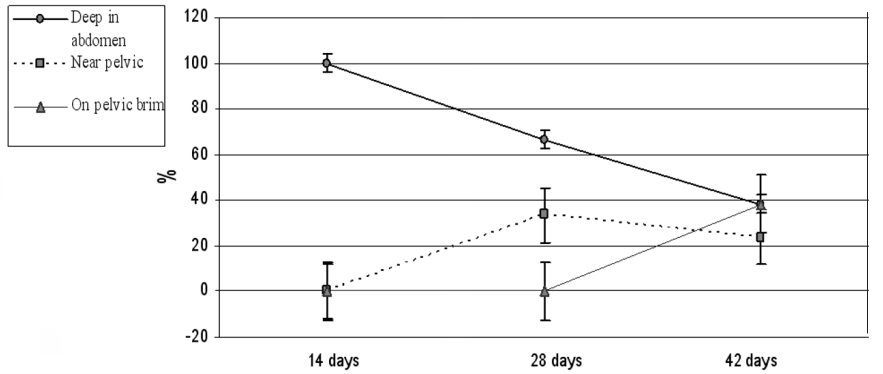

Fig. 3. Location of uterus at 14, 28, and 42 days post-treatment

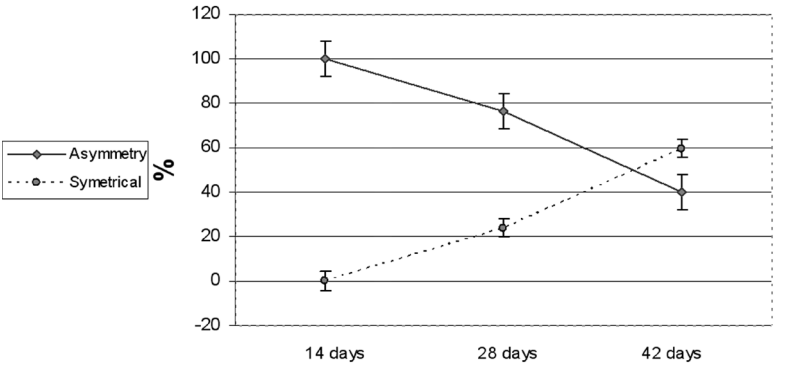

Fig. 4. Symmetry of uterine horns at 14, 28, and 42 days post-treatment

40.9 ± 0.18 °C, pulse rate 112.7 ± 8.33/ min., and respiration rate 52.3 ± 4.7/min, which were significantly higher ($P < 0.05$) than the control group. On vaginal examination there was a watery fetid bloody-brownish discharge. Their genital tracts when examined per rectum, 78.57% of buffalo cows with toxic puerperal metritis had a cervical diameter of more than 7.5 cm with non-retractable highly enlarged asymmetrical horns and the uterine walls were atonic and without signs of involution.

The variety and prevalence of bacterial isolation from buffalo cows with toxic puerperal metritis are listed in Table 1. Between the isolates, *E. coli* (18.52%), *Archanobacterium pyogenes* (16.67%), *Staphylococcus aureus* (12.96%) and *Fusobacterium necrophorum* (9.26%) were the most prevalent isolates. In the control group *E. coli* was the dominant bacteria in the uterus. All the isolates recovered from the uterus of buffalo cows with toxic puerperal metritis were mixed isolates and no single isolate was found from the uterus.

Histopathological examination of the endometrium showed acute endometritis associated with massive infiltration of multinucleated inflammatory cells around the uterine glands and blood vessels, hyperemia in blood vessels with hemorrhage in different sites of stroma. The percentages of polymorphonuclear cells were significantly higher ($P<0.01$) in buffalo cows with toxic puerperal metritis compared to the control group.

Fig. 1. describes the results of sensitivity tests of various bacterial isolates from the genital tract of buffalo cows affected with toxic puerperal metritis. These results revealed that rifampicin and oxytetracycline were sensitive to most bacterial isolates, 73.8% and 67.9%, respectively. Results of susceptibility tests revealed the resistance rates to

erythromycin (63.1%), amoxicillin (59.2%), penicillin (78.6%) gentamycin (68.8%) and streptomycin (41.8%) and they were the drugs mostly resisted by the isolated bacteria.

Table 2 describes the treatment effect on the percentage of polymorphonuclear cells after 14 and 28 days post-treatment, as well as the character of discharge. Sixteen buffaloes received a second dose of the same treatment after 14 days of the first treatment due to persistence of purulent discharge on vaginal examination. Results indicated a high clinical cure rate of buffalo cows affected with toxic puerperal metritis using rifampicin as an intrauterine infusion, and oxytetracycline systemically with estradiol and oxytocin, as 61.9% had clear secretions after 28 days post-treatment, while 16 were still having a purulent discharge (38.1%) and the fetid smell of the vulval discharge disappeared in all buffalo cows treated after 14 days. The PMNs were significantly decreased ($P < 0.01$) when compared with PMN before treatment. Results of recording uterine involution are shown in Figs. 2-4. Cervical involution was completed in 40% in buffaloes after 42 days of treatment. The location of the uterus on the pelvic brim was nearly complete in all treated buffaloes, while 60% of the uterus of treated buffaloes became symmetrical after 42 days of treatment.

Discussion

Archanobacterium pyogenes and *E. coli* with many others like *Staphylococcus* spp*., Streptococcus* spp*., and Proteus* spp. are frequently isolated from cows with retained placenta and cows with acute metritis (LOHUIS et al., 1994). Results indicated that *E. coli*, *Archanobacterium pyogenes*, *Staphylococcus aureus* and *Fusobacterium necrophorum* are mainly present in a diseased postpartum uterus. From bacteriological studies of toxic puerperal metritis of buffalo cows included in this study, it can be suggested that uterine inflammation occurs as a result of postpartum ascending contamination by non-specific environmental organisms. Periparturent insults, including dystocia, uterine prolapse, and retained fetal membranes diminish uterine ability to eliminate contaminated organisms.

Oxytetracycline, selected for this study, was already used for the treatment of uterine infection in dairy cattle. To choose an appropriate antibiotic to treat uterine infection, it is essential to know the susceptibility and resistance of antimicrobial agents available for the principal pathogens isolated from the uterus. In vitro susceptibility tests are generally useful for predicting the clinical outcome of an antimicrobial treatment, and then allow clinical studies to focus on the antibiotics likely to be successful in vivo, thus avoiding the unnecessary and unethical treatment of diseased animals with compounds that are likely to be ineffective. However, they are limited by their ability to take account of factors such as the drug's ability to penetrate the host's infected tissue, the contribution of the host's immune defense and ability of antibiotics to kill bacteria. Although oxytetracycline is widely used for the treatment of uterine infection in dairy cattle, there was evidence of

resistance in some of the bacteria. Among the 20 strains of *E. coli,* 4 (20%) showed high-level resistance to oxytetracycline. The total percentage of resistance of all bacteria isolated from the uterus of diseased buffalo cows to oxytetracycline was 22.1%. This resistance is invariably due to inheritance of a plasmid, encoded efflux mechanism (FOSTER, 1983) which should be expected to resist oxytetracycline therapy. The results underline the relative inefficiency of oxytetracycline against *Archanobacterium pyogenes,* as six strains (80%) were susceptible, and four resistant (20%). These results were in agreement with COHEN et al. (1995) and SHELDON et al. (2004b). However, *Archanobacterium pyogenes* shows the highest susceptibility to rifampicin, as 18 strains were sensitive. The most effective drug was rifampicin (73.8 %) followed by oxytetracycline (67.9 %). These results suggested that these drugs should be considered for the treatment of uterine bacterial infection in buffaloes but resistance to these agents among bacteria was not uncommon.

The cure responses of toxic puerperal metritis of buffaloes after intrauterine infusion with rifampicin and systemic injections of oxytetracycline, are seen by comparing PMN percentage before treatment, as well as improvement of the uterine involution. Higher concentrations throughout the genital tract are achieved with systemic administration than with intrauterine therapy of oxytetracycline (BRETZLAFF, 1986; OTT et al., 1986). Systemic treatment is best if antibiotics are subjected to degradation by conditions in the uterine lumen. Animals with toxic puerperal metritis have an invasion of microorganisms into deeper layers of the uterine wall (DRILLICH et al., 2001). This would imply that effective concentrations of antibiotic are desirable in all regions of the genital tract. BRETZLAFF et al. (1983) found no significant differences in blood plasma and that of genital tissue ratio of oxytetracycline when administered systemically early postpartum. While following intrauterine infusion into early postpartum cows with toxic puerperal metritis was associated with decreased drug absorption. This was reflected in reduced plasma concentration and therefore lowers tissue concentration of oxytetracycline in the sub endometrial tissues (BRETZLAFF et al., 1983). In addition, recent studies recommend the usage of oxytetracycline in the systemic treatment of cows affected by toxic puerperal metritis (DRILLICH et al., 2001; LEBLANC et al., 2002; SHELDON et al., 2004b). Evacuation of the uterus contributes to the success of further antibiotic therapy (ROBERTS, 1986). Further studies are needed to see the pharmacodynamics and pharmacokinetics of rifampicin in the female reproductive system. Evacuation can be hastened by use of estradiol benzoate and oxytocin to expel the fluid or hasten onset of estrus (NOAKES et al., 2002). When fluids have been expelled, the effectiveness of antibiotics in clearing the remaining infection is improved. Estradiol benzoate administered to buffaloes with toxic puerperal metritis could enhance the effect of oxytocin by stimulating the synthesis of endometrial and myometrial oxytocin receptors (FUCHS et al., 1990). Oxytocin after 12 h of the estradiol injection, increases the frequency and amplitude of myometrial contractions and indirectly, through the local endometrial, release of prostaglandin F2α

(ROBERTS et al., 1976; TAVERNE et al., 2001). The results indicate that sequential estradiol and oxytocin application with antibiotics in buffalo cows affected with toxic puerperal metritis positively effected a cure and uterine involution. However, more studies on a large number of animals are needed to confirm the practical usefulness of estradiol and oxytocin and antibiotic treatment of toxic puerperal metritis and its effects in improving fertility.

Conclusions

Intrauterine infusion of rifampicin and systemic injection of oxytetracycline and sequential application of estradiol benzoate and oxytocin positively effected a cure and uterine involution in buffaloes with toxic puerperal metritis.

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SAŽETAK

Svrha ovoga istraživanja bila je procijeniti učinkovitost upotrebe antibiotika u kombinaciji s određenim hormonalnim preparatima za liječenje toksičnoga metritisa u bivolica. Uzorci su bili uzeti od 42 bivolice s toksičnim puerperalnim metritisom na dvije velike bivolje farme. Obrisci za bakteriološku i bioptati za patohistološku pretragu bili su uzeti sa sluznice maternice bivolica s puerperalnim metritisom. Najčešće izdvojene bakterijske vrste iz maternične šupljine bile su *Escherichia coli*, *Archanobacterium pyogenes*, *Staphylococcus aureus* i *Fusobacterium necrophorum*. Većina bakterijskih izolata bila je osjetljiva prema rifampicinu i oksitetraciklinu odnosno 73,8% izolata bilo je osjetljivo prema rifampicinu, a 67,9% prema oksitetraciklinu. Liječenje bivolica rifampicinom i oksitetraciklinom dovelo je do kliničkog ozdravljenja i involucije maternice. Zaključuje se da je primjena estradiol benzoata i oksitocina s intrauterinom infuzijom rifampicina i sistemskom primjenom oksitetraciklina pozitivno djelovala na kliničko izlječenje i involuciju maternice u bivolica s toksičnim puerperalnim metritisom.

Ključne riječi: bivolica, toksični puerperalni metritis, bakterije, oksitetraciklin, rifampicin