

Evaluation of therapeutic effects of a 5-day intrauterine infusion of 6% *Eucalyptus globulus* oil solution on mares with experimentally induced endometritis with *Streptococcus zooepidemicus*

Gharagozlou, F.¹; Ghasemzadeh-Nava, H.¹; Atyabi, N.¹;
Sharifi Yazdi, H.² and Akbarinejad, V.^{3*}

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ²Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; ³Young Researchers and Elites Club, North Tehran Branch, Islamic Azad University, Tehran, Iran

*Correspondence: V. Akbarinejad, Young Researchers and Elites Club, North Tehran Branch, Islamic Azad University, Tehran, Iran. E-mail: v_akbarinejad@ut.ac.ir

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Summary

Endometritis is one of the major causes of infertility in mares. The present study was conducted to evaluate intrauterine infusion of *Eucalyptus globulus* oil as treatment for mares with experimentally induced endometritis. Mares (n=8) were synchronized using two injections of PGF₂α (10 mg) 14 days apart. All mares were inoculated with a live culture of 5×10^6 CFU *S. zooepidemicus* in the dioestrus phase after the termination of oestrus. The day of inoculation was considered as day 0 of the experiment. On days 3, 6 and 9, cytological and bacteriological specimens were collected to determine endometritis. Mares were randomly assigned to two groups on day 10. Mares in treatment (n=4) and control (n=4) groups received intrauterine 6% *Eucalyptus* oil solution (100 ml) and intrauterine saline solution (100 ml) for 5 consecutive days. Cytological, bacteriological, and ultrasonographic evaluations were implemented at days 15, 17 and 19 (1, 3 and 5 days after termination of intrauterine treatment, respectively). The number of mares diagnosed with endometritis, the depth of their intraluminal uterine fluid and the number of infected mares did not change by the treatment ($P>0.05$). In conclusion, the 5-day intrauterine infusion of 6% *E. globulus* oil solution failed to treat experimental endometritis in mares.

Key words: Mare, Endometritis, *Eucalyptus* oil, Intrauterine therapy

Introduction

Endometritis is one of the major causes of infertility in mares, resulting in significant economic problems for the equine industry (Watson, 2000; Hurtgen, 2006; Liu and Troedsson, 2008). Different organisms have been isolated from mares with uterine infection including *S. zooepidemicus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* (Causey, 2006). Among these, *S. zooepidemicus* is considered to be the major cause of endometritis (Casagrande *et al.*, 2011). Uterine lavage, the use of echolic agents and antibiotic therapy have been suggested as main treatments for endometritis (Liu and Troedsson, 2008). Other therapies including intrauterine chelators (LeBlanc and Causey, 2009), mucolytics (LeBlanc and Causey, 2009; Witte *et al.*, 2012), corticosteroids (Bucca *et al.*, 2008; Wolf *et al.*, 2012) and immunomodulatory agents (Rohrbach *et al.*, 2006; Rogan *et al.*, 2007; Christoffersen *et al.*, 2012) have also been reported to be effective in the treatment of equine endometritis.

Eucalyptus oil components, particularly 1,8-cineole, have antibacterial, antiviral, antifungal and antiparasitic effects (Sadlon and Lamson, 2010). Moreover, *Eucalyptus* oil has been reported to act as an immunomodulatory (Serafino *et al.*, 2008) and anti-inflammatory agent (Juergens *et al.*, 2004). Accordingly,

the present study was conducted to investigate the therapeutic effects of the intrauterine treatment of *E. globulus* oil on mares experimentally infected with *Streptococcus zooepidemicus*.

Materials and Methods

Experimental design

Mares (n=8) confirmed to be healthy based on cytological, bacteriological and ultrasonographic evaluations were selected for the present study. To synchronize oestrous cycles, mares received two injections of PGF₂α (10 mg, Vetalyze, Aburaihan, Iran) 14 days apart. Expression of behavioral oestrus commenced approximately 3 days after the last PGF₂α administration. Upon termination of behavioral oestrus, mares were subjected to ultrasonography to confirm the presence of *Corpus luteum* as the indicator of the dioestrus phase. All mares were inoculated with a live culture of 5×10^6 CFU *S. zooepidemicus* in the dioestrus phase. The day of inoculation was considered as day 0 of the experiment. On days 3, 6 and 9, cytological and bacteriological specimens were collected and evaluated to determine endometritis. Mares were randomly assigned to two groups on day 10. Treatment (n=4) and control (n=4) groups received intrauterine 6% *Eucalyptus* oil solution (100 ml, Barij

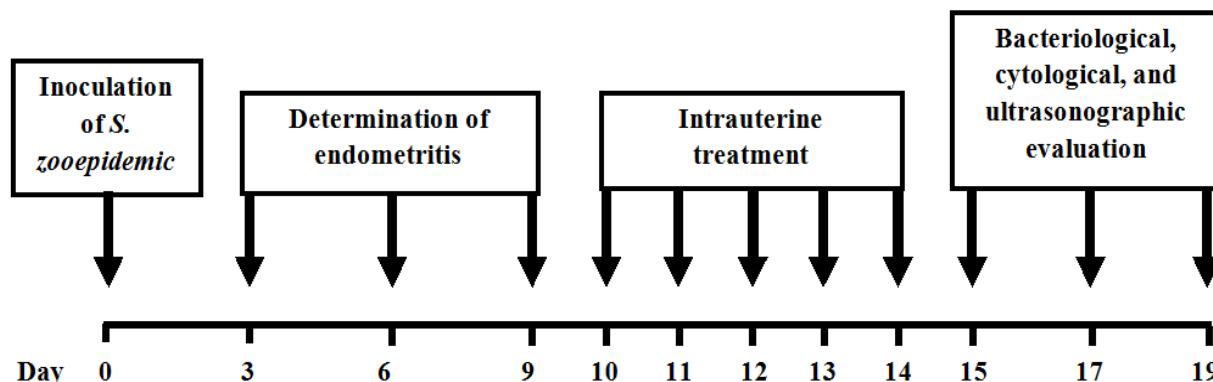


Fig. 1: Experimental design. Mares in treatment (n=4) and control (n=4) groups received intrauterine 6% *Eucalyptus* oil solution (100 ml, Barij Essence, Iran) and intrauterine saline solution (100 ml), respectively, on days 10, 11, 12, 13, 14

Essence, Iran) and intrauterine saline solution (100 ml), respectively. Treatment with either *Eucalyptus* oil or saline solution started on day 10 and continued for 5 consecutive days (Fig. 1).

Cytology

Specimens collected from the uterine body using guarded protective swabs on days 15, 17 and 19 (1, 3 and 5 days after the termination of intrauterine treatment) were rolled on glass slides, dried at room temperature, and stained with giemsa stain. Samples were viewed under $\times 40$, and polymorphonuclear cells were counted and averaged in 10 fields of view (Fig. 1). Mares with an average of >4 polymorphonuclear cells per field were classified as positive for endometritis (Rogan *et al.*, 2007).

Bacteriology

Endometrial specimens were collected using guarded swabs on days 15, 17 and 19 (1, 3 and 5 days after the termination of intrauterine treatment) and cultured on 5% sheep blood agar at 37°C for 48 h to determine the presence (positive) or absence (negative) of *S. zooepidemicus* (Fig. 1). Mares with positive bacteriological results were considered as infected and those with negative bacteriological results were considered as non-infected.

Ultrasonography

Intraluminal uterine fluids were visualized using transrectal ultrasonography on days 15, 17 and 19 (1, 3 and 5 days after the termination of intrauterine treatment), and the depth of the widest part was recorded for each mare (Fig. 1).

Statistical analysis

Changes in the depth of intrauterine fluids over time were analyzed using the GLM procedure including repeated measures in the model. The proportion of mares diagnosed with endometritis and the proportion determined as infected 1, 3 and 5 days after the treatment (days 15, 17 and 19 of experiment) were analyzed using Genmod procedure including logit in the model. All analyses were conducted in SAS (2001).

Results

Based on cytological results, the treatment did not influence the proportion of mares diagnosed with endometritis 1, 3 and 5 days after treatment ($P>0.05$, Fig. 2). The treatment had, therefore, no effect on the

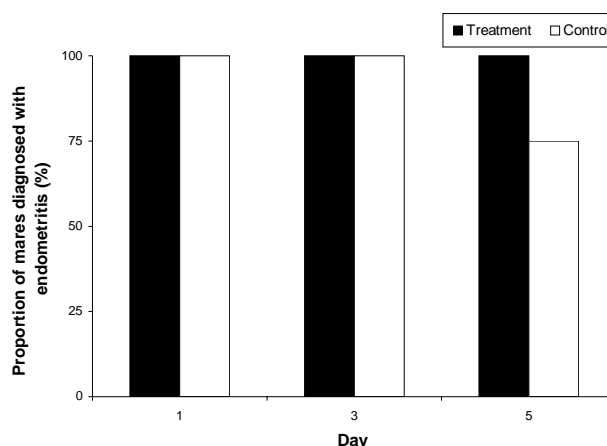


Fig. 2: Proportion of mares (%) diagnosed with endometritis, based on cytological results, 1, 3 and 5 days following treatment (days 15, 17 and 19 of experiment) in treatment and control groups

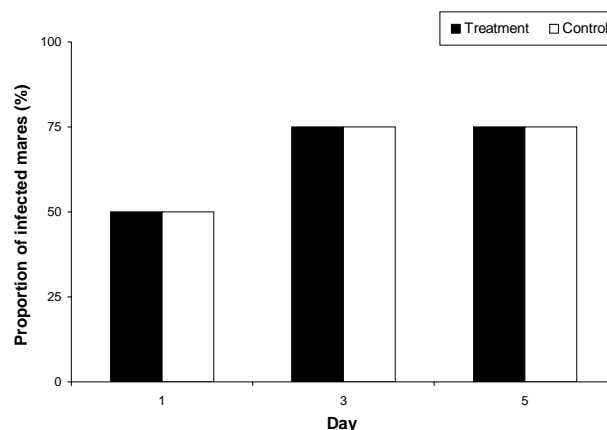


Fig. 3: Proportion of mares (%) infected with *Streptococcus zooepidemicus*, based on bacteriological results, 1, 3 and 5 days following treatment (days 15, 17 and 19 of experiment) in treatment and control groups

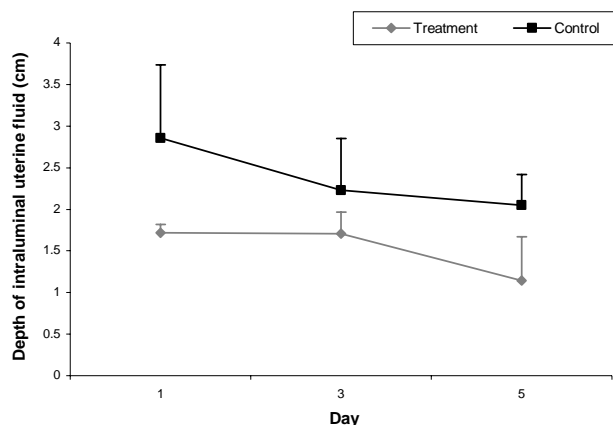


Fig. 4: Mean (\pm SEM) depth of intraluminal uterine fluid 1, 3 and 5 days following treatment (days 15, 17 and 19 of experiment) in treatment and control groups

proportion of mares determined as infected based on bacteriological results 1, 3 and 5 days after treatment ($P>0.05$, Fig. 3). Treatment and treatment \times time did not affect the depth of intraluminal uterine fluid ($P>0.05$); however, time tended to influence the depth of intraluminal uterine fluid ($P=0.098$, Fig. 4).

Discussion

Although *Eucalyptus* oil has been effectively adopted for treatment of asthma, rhinosinusitis, and chronic obstructive pulmonary disease in humans (Sadlon and Lamson, 2010), and has been shown to significantly reduce the severity of bronchitis symptoms in rats (Sadlon and Lamson, 2010), in the present study, the 5-day intrauterine infusion of 6% *E. globulus* oil solution failed to eliminate equine endometritis.

In the present study, treatment with *Eucalyptus* oil failed to reduce *S. zooepidemicus* infections in the mares. Testing the antimicrobial activity of essential oils of *Eucalyptus sideroxylon* and *Eucalyptus torquata* on nine bacterial species, Ashour (2008) found that all four assessed Gram-positive species (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Enterococcus faecalis*, and *Bacillus subtilis*) were highly sensitive, but only two (*Klebsiella pneumoniae* and *Proteus mirabilis*) out of five Gram-negative bacteria were sensitive. On the other hand, evaluation of the sensitivity of bacterial specimens isolated from humans to *Eucalyptus globulus* oil in the study conducted by Prabuseenivasan *et al.* (2006) revealed that some Gram-positive species such as *Streptococcus pneumoniae* and *Streptococcus agalactiae* were sensitive, while others such as *Streptococcus pyogenes* and *Staphylococcus aureus* were slightly sensitive. Furthermore, *E. globulus* oil had no effectiveness against *Klebsiella pneumoniae* (Prabuseenivasan *et al.*, 2006). Therefore, it seems that sensitivity to *Eucalyptus* oil could be dependent on either bacteria species and isolates or the *Eucalyptus* species itself. Accordingly, in the present study, the failure of the intrauterine treatment with *Eucalyptus* oil could have been partly originated from the insensitivity of the *S.*

zooepidemicus used to induce endometritis to *E. globulus* oil. In this context, the effects of other *Eucalyptus* species need to be evaluated as treatment for equine endometritis.

Regardless of the effect of bacteria and *Eucalyptus* species, it could also be speculated that the dose and/or duration of treatment might have affected the therapeutic effects of *Eucalyptus* essential oils. In this context, doses $>6\%$ of *E. globulus* oil and/or a treatment of more than 5 days might serve as an effective therapy for equine endometritis. Therefore, further studies on dose and duration of intrauterine treatment with *Eucalyptus* essential oil seem necessary.

Regardless of the treatment, the depth of the intrauterine fluid decreased over time in all mares, implying the spontaneous elimination of uterine fluids. Uterine contractility is a defense mechanism, contributing to the clearance of fluids from the uterus (Nikolakopoulos and Watson, 1999). Mares with contractile defects of the myometrium are susceptible to endometritis (Rigby *et al.*, 2001). Moreover, the reduction of uterine contractility using clenbuterol in mares with endometritis has been demonstrated to lead to the accumulation of intrauterine fluid (Nikolakopoulos and Watson, 1999).

In conclusion, the 5-day intrauterine treatment with 6% *E. globulus* oil solution failed to treat mares which were experimentally induced with endometritis by *S. zooepidemicus*.

References

- Ashour, HM (2008). Antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of *Eucalyptus sideroxylon* and *Eucalyptus torquata*. *Landes Biosci.*, 7: 399-403.
- Bucca, S; Carli, A; Buckley, T; Dolci, G and Fogarty, U (2008). The use of dexamethasone administered to mares at breeding time in the modulation of persistent mating induced endometritis. *Theriogenology*. 70: 1093-1100.
- Casagrande, PP; Bietta, A; Coppola, G; Felicetti, M; Cook, RF; Coletti, M; Marenzoni, ML and Passamonti, F (2011). Isolation and characterization of β -haemolytic-Streptococci from endometritis in mares. *Vet. Microbiol.*, 152: 126-130.
- Casey, RC (2006). Uterine therapy for mares with bacterial infections. In: Samper, JC; Pycock, JF and McKinnon, AO (Eds.), *Current therapy in equine reproduction*. (1st Edn.), St. Louis, Missouri, Saunders Elsevier. PP: 105-115.
- Christoffersen, M; Woodward, EM; Bojesen, AM; Petersen, MR; Squires, EL; Lehn-Jensen, H and Troedsson, MH (2012). Effect of immunomodulatory therapy on the endometrial inflammatory response to induced infectious endometritis in susceptible mares. *Theriogenology*. 78: 991-1004.
- Hurtgen, JP (2006). Pathogenesis and treatment of endometritis in the mare: a review. *Theriogenology*. 66: 560-566.
- Juergens, UR; Dethlefsen, U; Steinkamp, A; Gillissen, A; Repges, R and Vetter, H (2004). Anti-inflammatory activity of 1,8-Cineole (Eucalyptole) in bronchial asthma: a double-blind placebo controlled trial. *Respir. Med.*, 97: 250-256.

- LeBlanc, MM and Causey, RC** (2009). Clinical and subclinical endometritis in the mare: both threats to fertility. *Reprod. Domest. Anim.*, (Suppl.), 3: 10-22.
- Liu, IKM and Troedsson, MHT** (2008). The diagnosis and treatment of endometritis in the mare: yesterday and today. *Theriogenology*. 70: 415-420.
- Nikolakopoulos, E and Watson, ED** (1999). Uterine contractility is necessary for the clearance of intrauterine fluid but not bacteria after bacterial infusion in the mare. *Theriogenology*. 52: 413-423.
- Prabuseenivasan, S; Jayakumar, M and Ignacimuthu, S** (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Comp. Alternat. Med.*, 6: 39-46.
- Rigby, SL; Barhoumi, R; Burghardt, RC; Colleran, P; Thompson, JA; Varner, DD; Blanchard, TL; Brinsko, SP; Taylor, T; Wilkerson, MK and Delp, MD** (2001). Mares with delayed uterine clearance have an intrinsic defect in myometrial function. *Biol. Reprod.*, 65: 740-747.
- Rogan, D; Fumuso, E; Rodriguez, E; Wade, J and Sánchez-Bruni, SF** (2007). Use of a mycobacterial cell wall extract (MCWE) in susceptible mares to clear experimentally induced endometritis with *Streptococcus zooepidemicus*. *J. Vet. Sci.*, 27: 112-117.
- Rohrbach, B; Sheerin, P; Steiner, J; Matthews, P; Cantrell, C and Dodds, L** (2006). Use of Propionibacterium acnes as adjunct therapy in treatment of persistent endometritis in the broodmare. *Anim. Reprod. Sci.*, 94: 259-260.
- Sadlon, AE and Lamson, DW** (2010). Immune-modifying and antimicrobial effects of *Eucalyptus* oil and simple inhalation devices. *Altern. Med. Rev.*, 15: 33-47.
- SAS** (2001). *Statistical Analysis System: A User's Guide*. Version 8.2, Cary, NC, USA.
- Serafino, A; Vallebona, PS; Andreola, F; Zonfrillo, M; Mercuri, L; Federici, M; Rasi, G; Garaci, E and Pierimarchi, P** (2008). Stimulatory effect of *Eucalyptus* essential oil on innate cell-mediated immune response. *BMC Immunol.*, 9: 17-32.
- Watson, ED** (2000). Post-breeding endometritis in the mare. *Anim. Reprod. Sci.*, 60-61: 221-232.
- Witte, TS; Melkus, E; Walter, I; Senge, B; Schwab, S; Aurich, C and Heuwieser, W** (2012). Effects of oral treatment with N-acetylcysteine on the viscosity of intrauterine mucus and endometrial function in estrous mares. *Theriogenology*. 78: 1199-1208.
- Wolf, CA; Maschwitzky, E; Gregory, RM; Jobim, MI and Mattos, RC** (2012). Effect of corticotherapy on proteomics of endometrial fluid from mares susceptible to persistent postbreeding endometritis. *Theriogenology*. 77: 1351-1359.