

## Effect of intrauterine *Allium sativum* extract on recovery and conception rate in dairy cows with subclinical endometritis

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### ABSTRACT

The current study was designed to investigate the therapeutic effectiveness of *Allium sativum* (garlic) extract infusion on recovery and conception rate in cows with subclinical endometritis. Cows (42) with subclinical endometritis were randomly divided into 2 equal groups. In treatment group (group T), cows received a single intrauterine administration of 10 ml garlic extract mixed with 40 ml saline. In the cows of control group (group C), no intrauterine treatment was administered. Samples were collected again from the all animals of both groups on day 14 after therapy and the same laboratory tests were repeated. The cows were artificially inseminated at the following estrus. 10 (23.8 %) out of 42 cows had subclinical endometritis without bacteria in the uterus; however majority of cows with subclinical endometritis were positive for bacterial infection (76.2%). Bacterial load in group T was significantly lower as compared to both pre-treatment and group C. The cure rate for group T and C was 100.0 and 19.0%, respectively. The conception rates in groups T and C were 52.4 and 14.3%, respectively. Our results indicated that the intrauterine use of garlic extract can decrease endometrial inflammation and bacterial load, and consequently an increase in the conception rate.

**Key words:** Cow, Garlic extract, Subclinical endometritis

Subclinical endometritis, an inflammation of uterus that reduces reproduction (Kaufmann *et al.* 2009, Sheldon *et al.* 2009) also described as ‘cytological endometritis’ (Gilbert *et al.* 2005), is the most prevalent one of all uterine diseases in lactating dairy cows. Incidence of subclinical endometritis varies from 12 to 94 % in cows (Kaufmann *et al.* 2009). Treatments such as prostaglandin F<sub>2</sub>α or analogues, intrauterine antibiotics and antiseptic solutions or proteolytic enzymes are used for cows suffering with subclinical endometritis, but these therapies are often not satisfactory (Meziane *et al.* 2013). In view of milk disposal after antibiotic treatment, the magnitude of the problem of drug resistance, a reduced phagocytic activity of polymorphonuclear leukocytes and the coagulation necrosis of the endometrium there is a need to find out an alternative approach for treatment of subclinical endometritis (Rahi *et al.* 2013). Use of plant extracts having antimicrobial and anti-inflammatory features can be of quite importance as therapeutic agents in subclinical endometritis (Rahi *et al.* 2013). Garlic has been used for thousands of years to treat many disease conditions. Garlic, *Allium sativum*, contains 33 sulphuric compounds (Mohammadzadeh *et al.* 2014). Sulfur compounds containing allicin are the primary effective ingredients in the root bulb of the garlic plant (Tattelman 2005). Allicin has properties, viz.

anticoagulation, antihypertensive, antibiotic, antiparasitic, antimycotic, antiviral, anti-tumoral, anti-oxidant, immunomodulator (Corzo-Martinez *et al.* 2007, Andleeb *et al.* 2014). Allicin realizes the effect owing to interrupting oxidation of thiol group of bacterial enzymes and impairment of synthesis of bacterial RNA, proteins and enzymes (Joe *et al.* 2009). Garlic has antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria and fungi (Eja *et al.* 2007). Because the form of influence of garlic is quite dissimilar from that of antibiotics, a creation of a resistance against garlic for most bacteria is quite difficult (Jabar and Al-Mossawi 2007). Intrauterine infusions of garlic extract was as effective as antibiotic solutions in treatment of endometritis (Sarkar *et al.* 2006). Thus, garlic may be effective as a potential alternative herbal medication for subclinical endometritis. On the other hand, in spite of the extensive literature clarifying the utility of garlic in treating infections, there is little or no work reported to estimate the benefit of garlic in treating subclinical endometritis. Therefore, the current study was designed to investigate the therapeutic effectiveness of garlic extract infusion on recovery and conception rate in cows with subclinical endometritis.

### MATERIALS AND METHODS

*Study area, animals, sample collection, and treatments:* This study was carried out on Holstein cows (42), 4–7 year-

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old, with subclinical endometritis from a commercial dairy farm in Elazığ province of Turkey. All the used methods in this work are conformed to the regulations of International Animal Ethics Committee. Subclinical endometritis was determined using endometrial cytology. Apparently, healthy cows (176) at week 6–8 post-partum were examined by endometrial cytology using uterine lavage. Recovered fluid was taken to the laboratory in 2 sterile plastic tubes and placed on ice in a portable cooler. The first tubes were centrifuged at 800 rpm for 5 min. A drop of sediments were placed onto a clean microscope slide, air-dried, fixed with methanol and then stained with Geimsa for 45 min. The amounts of neutrophil were observed under light microscope at 40 $\times$ . The per cent of polymorphonuclear cells (PMN %) in the total number of cells counted was calculated. Finally, samples with greater than or equal 5 neutrophil were categorized as subclinical endometritis whereas amount of neutrophil less than 5 considered as normal (Moges and Jebar 2012, Gilbert *et al.* 2005).

The second tubes of cow with subclinical endometritis for bacteriology were cultivated in the blood agar media plates. The inoculated blood agar plates were incubated for 24 h at 37°C. The bacterial isolates grown in the blood agar media plates were identified by employing Cowan and Steel methods (1973).

The garlic used was purchased from local market. Garlic extract was prepared as per method outlined by Eja *et al.* (2011). Cloves of garlic were decorticated, cut in small pieces put in a juice extractor and pressed. The obtained extract (10.5 ml) was placed in a preweighed glass dish and reweighed, followed by drying in an oven at 60°C. Differences between the weight of the extract before and after drying were regarded as the amount of the active component of the extract which could have evaporated. The amount obtained for garlic was 1.12 mg. One ml of extract contained 106.7 mg of garlic materials. The obtained garlic extract was put in sterile screw cap bottles and stored in the refrigerator at 4°C for later use.

Totally, 42 cows with subclinical endometritis were diagnosed on the basis of presence of > 5% PMN cells in uterine cytological examination. After evaluation, these cows were randomly divided into 2 groups with 8 cows in each group as a treatment group (group T) and a control group (group C). All the animals were kept under the similar feeding and management conditions during experiment. The group T cows received a single intrauterine administration of 10 ml garlic extract mixed with 40 ml saline. The cows in group C received no treatment. Samples were collected again from the all animals of both groups on day 14 after therapy and the same laboratory tests were repeated. Cows were artificially inseminated at the following estrus. Pregnancy was checked by rectal palpation at 60 days post insemination.

**Data and statistical analysis:** Cure of subclinical endometritis from first examination to second examination was defined as a cow that had  $\geq$  5% PMN at first examination but that had < 5% PMN at second examination.

The < 5% PMN cut-off was based on Gilbert *et al.* (2005) reporting that a PMN % of < 5 at 40 to 60 day postpartum did not have a negative impact on reproductive outcomes. The outcome of the treatment was assessed by cytological cure rate defined as amount of neutrophil less than 5 at the re-examination after treatment, and by bacteriological cure and by conception rate. Pregnancy status at 60 days was used for the analysis.

The data so generated were analyzed using Chi-square test to test the significant differences of means. Statistical significance was taken to be indicated by  $P < 0.05$ .

## RESULTS AND DISCUSSION

The prevalence of subclinical endometritis is quite variable among studies, perhaps owing to different methods used to classify the disease. The incidence of subclinical endometritis ranged from 19 to 74 % in cows, and to be 53% on average (Gilbert *et al.* 2005, Plöntzke *et al.* 2010). In the present study, 176 lactating dairy cows which were normal clinically were screened for subclinical endometritis at week 6–8 post-partum, of which 42 cows (23.9%) were diagnosed with subclinical endometritis. The 23.9 % prevalence of subclinical endometritis in this study found at the first cytological examination falls within the range of 15.8–53% cited in the flush cytology (Barlund *et al.* 2008, Gilbert *et al.* 2005). Nevertheless, some researcher (Carneiro *et al.* 2014, Gilbert *et al.* 2005) found a higher incidence than observed in this study, while a lower incidence was reported by Madoz *et al.* (2013).

Bacterial infection of the uterus induces an inflammatory response in the uterus (Kaufmann *et al.* 2009). However, all cows with inflamed uterus might not develop uterine infection (Rahi *et al.* 2013). Ricci *et al.* (2015) detected uterine inflammation in the absence of bacteria where not all cows with detectable uterine inflammation (PMN %) were culture-positive for bacteria. In present study, 10 (23.8 %) out of 42 cows had subclinical endometritis without bacteria in the uterus; however majority of cows with subclinical endometritis were positive for bacterial infection (76.2%); 32 out of 42 cows (76.2%) yielded single (9 samples) and mixed type (23 samples) bacterial isolates including *E. coli*, *Staphylococcus* spp., *Streptococcus* spp. and *Bacillus* spp. in the pre-treatment uterine flushes. Gani *et al.* (2008) recorded similar reports of isolation of bacteria from the genital tract of sub-fertile cows.

In group T, 18 out of 21 (85.7%) cows had sterile uterine flushes on day 14 after garlic treatment; in the rest 3 (14.3%), the pre-treatment microbes persisted (*Staphylococcus* spp.). In group T, treatment with garlic extract reduced both the prevalence of positive uterine culture and subclinical endometritis in cows. In group C, bacterial isolates at second examination were similar to the first examination. A significant decline ( $P < 0.01$ ) in bacterial load was observed in group T from pre treatment to post treatment. After treatment, bacterial loads in groups T were significantly lower ( $P < 0.001$ ) as compared to group C. This observation is in agreement with several reports (Sarkar *et*

al. 2006, Rahi *et al.* 2013). It is reasonable to assume that this response may play an important role in controlling bacterial infection in subclinical endometritis of garlic extract. This effect could be due to the antimicrobial property against Gram positive and Gram negative bacteria of garlic extract (Eja *et al.* 2007) as well as strengthening of natural uterine defense mechanism (Schäfer and Kaschula 2014). Allicin acts by totally inhibiting RNA synthesis and partially inhibiting DNA and protein synthesis, suggesting that RNA is the primary target of allicin (Feldberg *et al.* 1988). Bacterial susceptibility to garlic might also be due to structural differences of the bacterial strains (Deresse 2010). The cell wall of Gram-negative bacteria contains 15–20% polysaccharides and 10–20% lipid, whereas Gram-positive bacteria contain 35–60% polysaccharides and only 0–2% lipid (Ikpeme *et al.* 2013). The polysaccharide and lipid contents of the cell wall have an effect on the permeability of allicin and other garlic constituents; this may be responsible for the difference in susceptibility to garlic between gram negative and gram-positive bacteria (Eja *et al.* 2007).

The PMN % at pre-treatment was similar among groups (8.6 and 8.8 for the group C and group T, respectively). On day 14 after therapy, PMN % declined significantly ( $P < 0.01$ ) in group T (1.5%) (Fig 1) and 52.4% of these conceived when inseminated. However, in group C the PMN % was not different significantly between the first or second examination. PMN cell count on day 14 after treatment in group T revealed a highly significant difference ( $p < 0.01$ ) as compared with both pretreatment and group C. Intrauterine garlic therapy reduced ( $P < 0.01$ ) PMN% on day 14 post-treatment than pretreatment levels. The cows were considered to have recovered from subclinical endometritis on day 14 after treatment on the basis of reduction in PMNs % ( $< 5\%$ ). In group T, of 21 re-examined cows with subclinical endometritis at first exam, 100% were found negative for subclinical endometritis cows at exam 2. At second exam of cows with subclinical endometritis in group C, 81.0% were diagnosed with subclinical endometritis. The cure rate in subclinical endometritis for group T and C was

100.0% and 19.0%, respectively. Among cows affected by cytological at first examination in group C, 19.0% had spontaneously cured at second examination. The recovery observed in group T may be due to antioxidant, broad spectrum antibacterial and good immunomodulatory properties of garlic (Guo *et al.* 2012) while the mild recovery in the control group could be attributed to natural uterine defense mechanisms leading to spontaneous recovery (Hoedemaker 1998). Garlic as an immunomodulator enhanced uterine defense mechanism resulting in enhanced phagocytosis of pathogen and natural healing with reduced denuded epithelium (Prasad *et al.* 2006). In addition to, garlic is natural antioxidant that helps the immune system fighting against the forming of free radical and foreign invasion including bacteria, virus (Rose *et al.* 2005). Hence, garlic might provide protection against oxidant induced damage with reduced oxidative reactions and enhanced antioxidant defense capabilities.

In group T, 52.4% of cow conceived when inseminated, in contrast to only 14.3% of cows in group C. The comparison of the conception rates for cows in groups C and T showed a statistically significant difference ( $P < 0.05$ ). Better conception rate observed in group T may be related to antioxidant and broad spectrum antibacterial and good immunomodulatory properties (Guo *et al.* 2012). The conception rate observed with treatment of garlic is in accordance with finding of Sarkar *et al.* (2006).

In conclusion, our results indicated that the intrauterine administration of garlic can decrease endometrial inflammation and may cause an increase in the conception rate. Moreover, as the garlic extract has antibacterial activity, it may be a good agent to avoid the use of antibiotics in the treatment of uterine infections. Garlic extract has an extraordinary potential to yield biologically active materials which could be valuable in the treatment of subclinical endometritis in cattle. Based on the results it was concluded that garlic extract may be recommended as an alternative therapy for subclinical endometritic cases.

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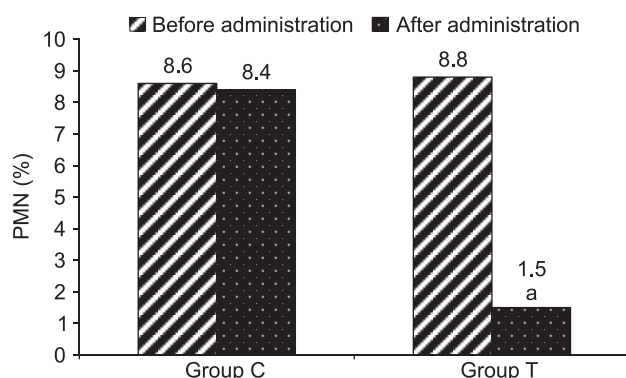


Fig. 1. Percentage of PMN in treated cows suffering from subclinical endometritis before and on day 14 after intrauterine administration of garlic and in controls. <sup>a</sup>Significant difference between pre- and post-treatment in group T ( $P \leq 0.01$ ).

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