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Effectiveness of *thymus vulgaris* essential oil on ovine mammary pustular dermatitis

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Abstract

The effectiveness of *Thymus vulgaris* L. Essential Oil (TvEO) for topical use in 21 sheep affected by mammary pustular dermatitis (MPD), not subjected to previous antibacterial therapy, was studied. After the evaluation of the antimicrobial susceptibility of isolated microorganisms (*Staphylococcus epidermidis* and *Staphylococcus aureus*) in milk samples to TvEO, some antimicrobials drugs (*amoxicillin-clavulanic acid*, *doxycycline*, *florfenicol* and *enrofloxacin*) and *Citrus bergamia* Risso et Poiteau EO (CvEO), the sheep, divided in two groups, were treated topically for 7 days with TvEO (13 animals) and CvEO (8 animals) respectively. The results showed that only TvEO induced a decrease in clinical signs 3 days after treatment and the complete disappearance of udder lesions after 7 days. No revival of infection was observed in the following months. The use of TvEO could be an alternative to antibiotic treatment in ovine affected by MPD

KeyWords: *Thymus vulgaris* EO, *Citrus bergamia* EO, mammary pustular dermatitis, ovine

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Introduction

In recent years, one of the most economically significant problems for a healthy sheep breeding is udder infections, widely diffuse in the world. Mastitis is one of the most common diseases in the dairy sheep, characterized by inflammatory and infectious processes, damage to mammary tissue and, consequently, reduced production and quality of milk. Common in ovine is also the mammary pustular dermatitis (MPD), aggravated by symptoms of mastitis, characterized by the appearance of pustules and vesicles, 2-4 mm in diameter, initially at the base of the teats which can then extend all over the skin of the udder. These lesions can develop into thick brownish irregularly circular scabs, which adhere to the underlying tissue. The udder appears enlarged, reddened, hot and painful on palpation, with decreased mammary secretion and altered chemical, physical and organoleptic characteristics of the milk. Diagnosis is made on clinical signs and by bacteriological testing of milk. The main pathogen responsible for mastitis in dairy sheep is *Staphylococcus aureus*, a Gram- positive organism,

cause of subclinical and chronic intramammary infections, often contagious.

Other microorganisms (staphylococci, streptococci, *E. coli*, mycoplasma, etc.) may also cause mammary infections associated with the animal's immunosuppression. Various are the factors predisposing to udder diseases: number of parts, udder conformation, lactation, hot-humid climate, incorrect milking, environmental hygiene, etc. Several different therapies have been used to fight the various bacterial infections responsible for mastitis and MPD in sheep and to solve the problem of bacterial resistance of several microorganism.

A valid alternative to traditional treatment, in order to counteract the development and spread of bacterial resistance, is represented by the use of essential oils (EOs), mixtures of organic substances, aromatic oils, biodegradable and with low toxicity, extracted from spices, aromatic herbs, fruit, flowers and plant material (*buds, seeds, leaves, twigs, bark, wood, roots*), effective natural products used against various infectious diseases (1, 2, 3, 4, 5).

Currently more than 3,000 EOs are known but only 300 are widely marketed. Some EOs obtained from *Thymus vulgaris*, *Zea mais*, *Melaleuca alternifolia*, *Citrus bergamia*, etc. are used in pharmaceuticals field because many studies report their antibacterial activity against many multi-resistant microbial species, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, ESBL *E. coli*, *Acinetobacter spp*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Bacillus cereus*, etc. (6, 7, 8, 9, 10). Other studies show that these EOs also have antifungal (11, 12, 13), antiviral (14), antioxidant, and anti-parasitic activity (15). Among EOs, *Thymus vulgaris* L. (TvEO) is used in dermatological, uro-gynecological and gastrointestinal diseases in human, for its beneficial properties due to presence of phenols, in particular, thymol (30% - 70%) and carvacrol (3% - 15%), recognized as anti-inflammatory, antiseptic, antispasmodic and vermicide substances, for external and internal use (16, 17, 18, 19 20). Some authors confirmed the antifungal activity of this EOs against *Candida albicans* isolated from bovine clinical mastitis (21, 22). In our previous investigations, the efficacy of TvEO for topical use in dog skin infections was evaluated (23).

The aim of the present study was to assess the effectiveness of *Thymus vulgaris* EO for topic use in ovine affected by MPD, aggravated by mastitis symptoms.

Material and methods

Sampling

From a clinical screening on 530 sheep from different farms in Messina, 21 animals affected by MPD aggravated by mastitis symptoms, not subjected to previous antibacterial therapy,

were selected and milk samples were taken before and 3, 5, 7 and 15 days after the EOs treatment.

Bacteriological investigations

The milk samples, after Gram staining, were enriched on BHI broth (Biolifes. Rl, Milan, Italy) for 18 hours at 37 °C and isolated on MacConkey-agar (Oxoid, Basingstoke, Hampshire, UK) and SM-Staphylococci 110 medium (Biolifes, Milan, Italy). After incubation for 24 hours at 37 °C, the isolated colonies were identified by Api System (bioMerieux SA, Marcy l'Etoile, France).

The *in vitro* susceptibility of isolated microorganisms to TvEO and CbEO (KOS S.r.l. Comeana-PO, Italy) and to some antimicrobial drugs widely used in ovine infection (*amoxicillin-clavulanic acid*, *doxycycline*, *florfenicol* and *enrofloxacin*) (Liofilchem S.r.l. Roseto degli Abruzzi – TE, Italy) was evaluated by Kirby-Bauer test.

Pharmacological treatment

The 21 animals were divided, *at random*, into two groups: the first group consisting of 13 sheep (n. 7 positive to *Staphylococcus epidermidis* and n. 4 positive to *Staphylococcus aureus*) and the second group consisting of 8 sheep (n. 4 positive to *Staphylococcus epidermidis* and n.4 positive to *Staphylococcus aureus*). Both groups were treated topically, by spraying the udder areas affected by lesions, for 7 days consecutively, with 4 ml (20 ml /L H₂O) of TvEO and CbEO respectively.

Clinical scores recorded before and after the treatment

The efficacy of TvEO was evaluated according to the presence of bacteria in the milk before, during and after the end of the treatment and on the basis of the scoring system relating to the regression of local clinical signs (*0 = absent; 1 = slight; 2 = limited; 3 = significant*): such as sensitivity to pain of the udder, increase in udder volume, presence and number of vesicles, pustules or scabs respectively.

Statistical analysis

The data were expressed as mean \pm S.D. elaborated using an analysis of variance (ANOVA). InStat 3.0 software (GraphPad Software San Diego, CA, USA) was applied to assess the significance of the difference between the scores recorded before and 3, 5, 7 and 15 days after the drugs treatment. Difference with $p < 0.05$ were considered significant.

Results

Bacteriological finding

Staphylococcus epidermidis (13 strains) and *Staphylococcus aureus* (8 strains) have been isolated from

milk samples. The *in vitro* susceptibility of isolated strains to EOs and some antimicrobials were reported in Table 1. We observed that *TvEO* is the most active antibacterial drug in this comparative analysis.

Pharmacological treatment

The bacteriological findings isolated from milk samples, before and 3, 5, 7 and 15 days after the end of treatment for 7 days with *TvEO* and *CbEO* respectively, were reported in Table 2

Table 1. *In vitro* susceptibility of Gram-positive microorganisms isolated from milk samples to *TvEO*, *CbEO* and to various antibacterial drugs.

DRUGS	Bacterial strains	
	<i>Staphylococcus epidermidis</i> (13)	<i>Staphylococcus aureus</i> (8)
<i>Thymus vulgaris EO</i>	+++	<i>Thymus vulgaris EO</i>
<i>Citrus bergamia EO</i>	++	<i>Citrus bergamia EO</i>
Amoxicillin-ac. clavulanic	++	Amoxicillin-ac. clavulanic
Doxycycline	+	Doxycycline
Florfenicol	-	Florfenicol
Enrofloxacin	++	Enrofloxacin

+++ Susceptible; ++ Intermediate; + Poorly susceptible; - Resistant

Table 2. Bacteriological findings isolated from milk samples before (T0 baseline), 3, 5, 7 and 15 days after topical treatment on ovine affected by MPD.

<i>Thymus vulgaris EO</i> (13 sheep)					
	T 0	3 days	5 days	7 days	15 days
<i>Staphylococcus epidermidis</i> (7)	++++	+++	+	-	-
<i>Staphylococcus aureus</i> (6)					
<i>Citrus bergamia EO</i> (8 sheep)					
<i>Staphylococcus epidermidis</i> (4)	++++	++++	+++	+++	+++
<i>Staphylococcus aureus</i> (4)	++++	+++	+++	+++	++

*The degree of positivity for the infection was expressed in the conventional manner (from + to +++) in relation to the quantity of bacteria present.

Clinical scores

The sheep affected by MPD at the end of *TvEO* treatment showed decrease in the clinical signs after 3 and 5 days (*the udder was less sensitive on palpation and less swollen*) and an evident complete remission after 7 days (Tab.3; Fig. 1). No local or general signs of *TvEO* intolerance were recorded in any treated ovine and no relapses occurred in the month following treatment. The treatment with *CbEO* (Group 2), instead, showed no significant effects.

Table3. EffectsoftopicaltreatmentwithTvEOfor7days(mean±S.D.)and scoring of clinical signs in ovine affected by MPD.

Clinical signs	Before treatment	After 3 days	After 5 days	After 7 days	After 15 ays
<i>Sensitivity to pain of the udder</i> (mean ±S.D.)	2.67 ± 0.18	**1.75 ± 0.46	*0.37 ± 0.12	-	-
<i>Increase in sudder volume</i> (mean ±S.D.)	2.84 ± 0.58	**1.48 ± 0.35	*0.42 ± 0.12	-	-
<i>Number of vesicles, pustules or scabs</i> (mean ±S.D.)	2.37 ± 0.51	**1.37 ± 0.51	0.17± 0.46	-	-

* $p < 0,01$; ** $p < 0,05$

Discussion

Our study documents the potential therapeutic efficacy of *TvEO* in ovine affected by mammary pustular dermatitis due to Gram-positive bacteria and showsan improvement in the clinical symptoms after 3 and 5 days of treatment with complete healing after 7 days. Furthermore, no local or general adverse reactions were observed during the various stages of therapeutic treatment. No therapeutic effects, instead, showed *CbEO* used in comparison as a positive control in ovine suffering from MPD, caused by Gram-positivebacteria.

The mechanism of antimicrobial activity of *TvEO* is quite complex and not been well elucidated.Some authors have observed that thymol, the main component of this oil,inhibits *Staphylococcus aureus*internalizationintobMECbyinhibitingNF-kBactivation (22).

However,being*Thymus EO* acomplex mixture,its antibacterial action at cellular level could be correlated not to a single mechanismbut probably to the synergic interaction of various organic compounds (19). The clinical studies reported in literature on EOs use in animal diseases are related mostly to *in vitro* study, for this reason it is very important to investigate their effectiveness and safety *in vivo*, as possible therapeutic alternative in veterinary medicine.

In one of our previous investigation *in vivo*, the therapeutic efficacy of *TvEO* in skin infections of dogs was documented (23). Currently there are no studies on the topical use of *TvEO* in ovine affected by MPD.

The results of our investigations show that the topical use of *TvEO*, also for its low cost, could constitute a possible alternative or a support to antibiotic therapy in dermatological infections, in particular in cases refractory to conventional therapy. This EO, in addition to showing a good antimicrobial activity, has other biological and therapeutic properties (antifungal, anti-inflammatory, antioxidant, etc.). In addition, it is believed extremely unlikely that bacteria can develop drug resistance to EOs, as these consists of several molecular components, compared to an antibiotic consisting of a single active substance(19). Moreover, it seems that EOs can affect synergistic activity with antibiotics, improving their therapeutic efficacy(4, 6).

Figure 1. Therapeutic efficacy of topic treatment with TvEO before and after 3 and 7 days in ovine affected by MPD



Conclusions

This study documents for the first time the effectiveness of *TvEO* for topical use in ovine affected by MPD. It is important to note that the follow-up with *TvEO* (7 days) is quite short as it is not an antibiotic and the speed of therapeutic effect is related to the high content of antioxidant substances, which possess antimicrobial, antifungal, anti-inflammatory effects, etc., as reported in literature. Therefore, the topical use of EOs on the skin and mucous membranes appears to be the most promising strategy to prevent or treat skin infections. However, further studies are needed, in particular on a greater number of cases and also on other diseases of farm breeding, to better evaluate therapeutic efficacy of *TvEO* in sheep.

Conflicts of interest: The authors declare no conflict of interest. Moreover, they have acquired the opinion of the Ethics Committee of Departement Veterinary Science (*Register number 033/19*).

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