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D 2A1.3: Operative management protocol for post-milking disinfection in cows and ewes using terpinen-4-ol as active principle (month 42)

Authors: ISS P. Aureli, AM Ferrini

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Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	X
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	



1. Introduction

The prevention of bovine mastitis is the most important component of a mastitis control program and both pre- and post-milking teat antisepses are one of the most effective procedures for preventing new intra-mammary infections (IMI) in dairy cows.

These procedures involve dipping teats of dairy cows before and/or after milking with appropriate germicidal preparations to reduce teat skin colonization and contamination both from contagious and environmental pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, *Corynebacterium bovis*, *Streptococcus uberis*, *Escherichia coli* and *Klebsiella pneumoniae*). Teat dipping is a simple, effective, and economical means to reduce bacterial populations on teat skin.

Of course, the occurrence of new IMI is related to the degree of teats' exposure to mastitis causing pathogens and to the number of bacteria on teat skin - especially near the orifice - but requires the penetration of mastitis causing microorganisms through the teat canal.

Pre-milking teat sanitation has been introduced to achieve an acceptable level of decontamination of teat skin by potential intra-mammary environmental pathogens, such as *Streptococcus uberis*, *Escherichia coli* and *Klebsiella pneumoniae*, on teat ends prior to attachment of milking machines. This results in reducing the spread of microorganisms and the occurrence of new IMI and - important from the food safety point of view - in minimizing the number of bacteria that find their way into the raw milk supply.

In addition, post-milking teat dipping is the most effective milking hygienic practice for preventing new infections caused by the two prevalent contagious mastitis microorganisms, *S.aureus* and *S.agalactiae*.

The overall effectiveness of post-milking disinfection has been improved by the use of protective coatings on the animals. Barrier dips which leave a germicidal film on the teats are a class of formulations designed to effectively reduce infection caused by environmental bacteria as well as reducing the spread of infections caused by contagious bacteria.

A variety of germicides can be incorporated into teat dip products such as iodine, chlorhexidine, quaternary ammonium, sodium hypochlorite, dodecyl benzene sulfonic acid, chlorine and so on. These germicides destroy bacteria



through chemical or biological action such as oxidation-reduction mechanisms, denaturation/precipitation of cytoplasmic proteins, inhibition of enzyme activity, and/or disruption of cell membranes.

Anyhow, in spite of all, mastitis remains one of the most serious diseases in veterinary productions causing the biggest economic losses to the dairy industry of developed countries. It impacts on animal production, animal health and welfare and the quality of the produced milk. Antibiotics play the major role in treating this infectious disease but their use exerts a strong pressure of selection, leading in the time to the development of resistant strains increasingly difficult to cure. Also human health concerns may be raised by milk consumption because of antibiotic residues in milk, transfer of antibiotic resistance from animal to human *via* zoonotic bacteria, and transfer of pathogens or products thereof through milk or milk products. As a consequence, the dramatic increase in antibiotic resistant pathogens requires the continuous need to find new agents to treat mastitis.

Present mastitis control strategy includes a combination of preventing measures (*i.e.* post-milking dipping and dry cow therapy) coupled with Good Veterinary Practice of Antimicrobials to promptly and appropriately prevent or treat new infections in a farm. Notwithstanding this, this control strategy is not always fully successful. Some evidences have indicated that the failure of mastitis control programs can be partly attributed to the teat disinfectants and/or antibiotics which do not afford sufficient protection against the numerous pathogens that cause mastitis particularly towards *Staphylococcus aureus* and *Streptococcus agalactiae*.

In this context, during the use, when a first choice antibiotic is not effective, a second one (more toxic and expensive) must be taken over, further contributing to maintain the antibiotic selective pressure in the environment. It is recognized that the progressive emergence of insensitive bacteria and of acquired resistance in human clinical setting and the veterinary fields reflects the “tuning of these microorganisms to antibiotic polluted eco-systems”.

2. Terpinen-4-ol post-milking teat dipping

Due to the potential of essential oils (and their components) to express antimicrobial activities, international literature is now ascribing a promising prospect to their application in human and veterinary medicine for their non-antibiotic nature.



Considering the prevalent strategy control program of mastitis and keeping in mind the characteristics of an ideal post dipping (wide antimicrobial spectrum, quick antimicrobial activity, non-skin irritating (physiological pH), stability, barrier effect, long persistence, no residue in milk) we have developed a preparation to apply for dipping based on a component of the *Melaleuca alternifolia* essential oil : the terpinen-4-ol.

The terpinen-4-ol (C₁₀H₁₈O; RTECS OT0175110 ; CAS NUMBER: 562-74-3; EC NUMBER (EINECS): 209-235-5) is a liquid, colourless to yellow, odour characteristic - mild earthy green with slight wood/pepper aspects, able to damage the microbial cell membrane.

We have demonstrated both *in vitro* and *in vivo* that a preparation containing terpinen-4-ol may be a profitable tool to combat antibiotic sensible and resistant agents of mastitis in milk producing animals - both of conventional and organic farms - with the aim to reduce the pressure for emergence and dissemination of antibiotic resistant strains in the environment.

The present deliverable describes a post-milking teat dipping formulated with terpinen-4-ol as active principle and its operative management protocol. This product was developed and tested *in vitro* in ISS and *in vivo* in an Italian farm and in the experimental plant of Marcenat (INRA-France) in the course of the WP2A activities.

2. 1. The composition:

Ingredients	% (w/w)
terpinen-4-ol	1-10
lower alkanols	5-10
gelling agent	0.2-5
distilled water	up to 100
dye	0.05
pH	5.0-6.0

Formula is coloured in blue (food grade colour) to recognize the treated teats and skin areas. A gelling agent included in the formula improves the persistence of the product while acting as a skin protective barrier.

It is a ready-to-use product with a high coating power for the sanitation of teats. Formula allows a complete protection of the orifice, is easy to use, gives no skin irritation, and presents a wide spectrum of antimicrobial activity. Its application causes no residue in the milk, so no withdrawal period is required



Several batches (6 Kilos/each) have been prepared in the course of the project to be used in the in vitro and in vivo experiments. Challenge tests were performed on each batch to check the antimicrobial activity. Formula was dispensed in non-transparent plastic containers of about 0.8-1 Kg/each. The containers used in the Italian farm experiment were labelled according to the public veterinary authorities requirements.

2.2. Operative management protocol

Although different procedures are reported for post-milking application, direct immersion of the teat in the formula seems to be the most effective modality as it prevents the operator from inhalation and exposure to the product and allows covering completely the teat skin with a thick coat of the product.

Post-milking formula is applied immersing animal teats in a cup containing the teat dip. For the application, the teat must be immersed in the formula for not less than 2-3 seconds. This procedure is satisfactory since it ensures the complete covering of the teat skin with the product in a very easy and quick way. Post-milking dip formula must be applied immediately after automated or manual milking on dry teats.

Formula is ready to be used and must be applied twice a day after each milking, covering at least 2/3 of the entire length of the teat.

During the use of the formula it is important to monitor regularly the udder health status using reports from the regional regulatory agency or milk marketing organization and from veterinary objective evaluations.

Packaged formula should be stored in areas avoiding extreme temperatures as freezing may cause separation of product components and overheating may volatilize ingredients and negatively affect the product efficacy. Containers in use must be kept closed to prevent contaminations.

Teat dip cups must be kept clean and sanitary; teat dip remaining in the cups should be never poured back in the original containers. Instead, contents must be used down to a low level periodically, discarding the remaining teat dip, and cleaning the cups thoroughly and refill with fresh dip.

If during milking, a product becomes grossly contaminated or diluted with milk, manure, other organic matter, or water, teat dip cup should be emptied, washed, and refilled with fresh product. In addition, cups should be washed after every milking or at least once a day.



Post milking dipping is not intended to replace or minimize good veterinary practices.

3. Patent application

An European Patent application of the formula has been applied (European Patent Appln. No. 08425431.7 filed on June 18, 2008 in the name of: ISS-INRA-IZSTLT) with the title: Disinfection and antimastitis use of terpinen-4-ol formula in milk producing animals.

Inventors: P. Aureli, Anna Maria Ferrini , Simonetta Amatiste, R Rosati, Marie-Christine Montel..