Innovative method for the treatment of mastitis in dairy animals

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AgNPs: Silver nanoparticles, ORP: Oxidation Reduction Potentials

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Abstract
The study was conducted in a herd consists of 800 buffaloes, in which 120 out of 160 clinically mastitic animals were selected to carry out the field study and to apply a novel strategy to control microbial infections in animal production. The mastitic buffaloes were having mixed infection with E. coli, Staphylococcus aureus and Streptococcus agalactiae. When applying 6 lines of treatment, the diseased animals were classified into 6 groups (20 each). The first group received local treatment by intramammary infusion of cefotiofur hydrochloride; the second one received systemic treatment of both enrofloxacin and carprofen; the third one received a combination of both local (cefotiofur) and systemic treatment (enrofloxacin and carprofen). The forth group received local treatment by intramammary infusion with 1:500 Envirolyte-Anolyte (2 mg of active chlorine). The fifth group received local treatment by intramammary infusion with AgNPs suspension. The sixth group received both local treatments with 1:500 Envirolyte-Anolyte (2 mg of active chlorine) and systemic treatment (enrofloxacin and carprofen). The cure rate was 60 % for the first group, 80 % for the second and third group. The fourth and fifth group both were 60 %, while 100% in the sixth group.

Citation:

1. Introduction
Economic losses were summarized by Barmely et al.(1986) and Varsheny & Naresh, (2004) including loss in milk production, discarded abnormal milk, degrading milk quality and price due to high bacterial or somatic cell count, cost of drugs, veterinary services, increased labor costs, increased risk of subsequent mastitis, herd replacement and problems related to antibiotics residues in milk and its products. Good quality starts on the farm by application of good hygienic measures to minimize the population of organisms on teats and udder (Fox et al., 2005).

The microorganisms responsible for most episodes of mastitis in dairy herds are the environmental pathogens (E. coli, Salmonella, Mycoplasma and Staphylococcus aureus (Cullor & Davis 2004). It is thought that contagious mastitis is primarily caused by S. aureus and Streptococcus agalactiae. Environmental mastitis can be caused by a variety of different bacteria, including, but not limited to, K. pneumoniae, Escherichia coli, Klebsiella oxytoaca, Enterobacter aerogenes, Streptococcus uberis, Streptococcus bovis, and Streptococcus dysgalactia.

Mastitis caused by Staphylococcus aureus is the most significant cause of economic losses of dairy industry; once it is established in the mammary gland, it is difficult to eradicate. To establish an infection process, it must overcome the different host defence mechanisms (phagocytosis, elimination by milking etc) (Aguilar et al., 2001).
Insufficient contact of the antibiotics with pathogenic bacteria at the site of infection is a major cause of mastitis treatment failure (Sandholm et al., 1990). The route of administration, intramammary or parenteral of medicinal products to treat mastitis is an important issue. It determines the biological barriers encountered by the active compound and the routes by which it may make contact with the causal microorganism (Serieys et al., 2005).

Traditional prevention of bovine mastitis involves a complex regimen of daily teat-dipping with a disinfectant solution, and may involve antibiotic-containing teat dips. When infection does occur, intramammary infusion of antibiotics is indicated; however this leads to increasingly resistant strains of bacteria.

Ceftiofur is a new broad-spectrum third generation cephalosporin antibiotics for veterinary use. It inhibits bacterial cell wall synthesis by interfering with enzymes essential for peptidoglycan synthesis (Hornish and Kotarski, 2002). Consequently, this new antibiotic should be effective against a wide variety of mastitis pathogens, including environmental mastitis pathogens (Oliver et al., 2004).

Therefore, it is necessary to discover novel strategies and identify new antimicrobial agents to develop the next generation of agents to control microbial infections in animal production.

2. Experimental

Traditional prevention of bovine mastitis involves a complex regimen of daily teat-dipping with a disinfectant solution, and may involve antibiotic-containing teat dips. When infection does occur, intramammary infusion of antibiotics is indicated; however this leads to increasingly resistant strains of bacteria.

2.1 Animal

This study was conducted in a farm at Alexandria Desert Road (a herd consists of 800 buffaloes) in which 120 out of 160 clinically mastitic animals were selected to carry out the field study.

2.2 Housing

Animals were housed in a separate yard stands on straw bedding floor, automatically milked twice daily and the milk collected in milk tank until treatment. They receive their needs of water through a common water trough.

2.3 Examination

A palpation evaluation was conducted for animals after complete milk out at the milking time according to Massart-Leen et al., (1988). Animals scored 1, showed pliable udder, heat pain, redness, and/or swelling were not detected; animals exhibited no signs of discomfort scored 2, where the udder was less pliable with some firmness, redness, heat, and pain were generally not detectable; animals exhibited no signs of discomfort scored 3. Animals exhibited moderate swelling udder, definitely firm, reddened, and warm to the touch and generally exhibited signs of discomfort (irritable, performed stepping motion with feet and/or kicked during the preparing and milking procedures) were scored 4. Udder showed sever swelling (the udder was very hard, red, hot, and noticeably larger than the other quarters) and of extremely uncomfortable and very irritable were scored 5. Udder was generally pliable and a hardened lump was palpable and present from milking to milking overtime without change in size, no pain, heat, redness, or swelling was associated with this condition scored 6. Udder was swollen, reddened, hard, and often extending forward toward the navel, as well as posteriorly up the rear quarters where the udder attaches to the body.

Appearance of foremilk was scored as follows:
1. Normal, 2. Flakes, 3. Small slugs, 4. Large slugs, 5. Stringy (watery), 6. Bloody. The mammary glands were considered to have clinical mastitis when the udder or milk score was 3 or higher.

2.4 Sampling

All samples were collected using standard procedures described by Harmon et al., (1990). After discarding the first few milk drops, milk samples were taken from all clinically mastitic buffaloes. In clean environment, thoroughly wiping the Teats and teat orifices were thoroughly wiped with 70 % ethyl alcohol. Each milk sample was collected in a sterile screw capped bottle and sent directly to the laboratory with minimum of delay for routine cultural identification.

All samples were kept on melting ice (1° C) during transport and at the laboratory until analysis were performed or aliquots were prepared for freezing (-20°C).

Milk samples were centrifuged at 300 rpm for 15 Minutes and a loopful from the sediment
and the rest of all samples were inoculated into a nutrient broth, brain heart infusion broth, then incubated aerobically overnight at 37°C for enrichment and enhancement of bacterial growth. Subcultures were streaked on 10% sheep blood agar and MacConkey agar plates according to Carter and Cole (1990). Suspected colonies were identified morphologically, microscopically and biochemically according to Quinn et al. (1994) and Waage et al. (1999). For Mycoplasma, a loopful was cultured on modified Hay flocks media, incubated at 37°C with 10% CO₂ for 7-10 days (Hogan et al., 1999). Specification of culture material was performed by an enzyme-linked immunosorbent assay (ELISA).

2.5 Treatment of Mastitic Cases

The test was performed according to Sayed and Abdel-Rady (2008). All diseased animals were classified into 4 groups (20 animals each). The first group received local treatment by intramammary infusion with 125 mg of ceftiofur hydrochloride (Pfizer Animal Health, Egypt); the second one received systemic treatment by I/M injection of both enrofloxacin 5 mg/kg body weight for 5 successive days and I/V injection of 2.9 ml/kg body weight of carprofen as an immunomodulator drug and the third one received a combination of both local (local intramammary infusion with ceftiofur) and systemic treatment (I/M injection of both enrofloxacin 5mg/kg body weight for 5 successive days and I/V injection of 2.9 ml/kg body weight of carprofen as an immunomodulator drug). The forth group received local treatment by intramammary infusion with 1:500 Envirolyte-Anolyte (It contains various mixed oxidants predominantly hypochlorous acid and sodium hypochlorite (HClO, ClO₂, HClO₃, HClO₄, H₂O₂, O₂, ClO⁻, ClO₂⁻, Cl⁻, O₂⁻, HO₂⁻, OH⁻) - working substances, (1:500) [pH 2.5-3.5, ORP>1150mV, Cactive~500mg/l], 1/500 = 2 mg/L active chlorine, 1/1000 = 1mg/L active chlorine.

The fifth group received local treatment by intramammary infusion with AgNPs suspension. The sixth group received both local treatments by intramammary infusion with 1:500 Envirolyte-Anolyte (2 mg of active chlorine) and received systemic treatment (I/M injection of both enrofloxacin 5mg/kg body weight for 5 successive days and I/V injection of 2.9 ml/kg body weight of carprofen as an immunomodulator drug).

The treatment applied once daily and for 5 successive days. Clinical cure was defined as the disappearance of clinical signs which were observed on day before treatment, on other words, by the return to normal feed intake, good general condition, absence of udder edema, normal milk appearance and normal milk yield.

Samples were taken from clinical mastitic quarters and examined bacteriologically to identify the causative agent.

3. Results and Discussion

Clinically mastitic cases were detected by clinically infected quarters often showing moderate swelling, firmness, visible signs of chunks of milk, clots in milk and in some cases, the milk become viscous. 120 out of 160 clinically mastitic buffaloes of mixed infection (E. coli, S. aureus, and S. agalactae) were selected to carry out the experiment.

Table 1: The efficiency of different lines of treatment which applied to cases of clinically mastitic buffaloes

![Table 1](image)

1: Local intramammary infusion with ceftiofur; 2: received systemic treatment by I/M in enrofloxacin and I/V carprofen, 3: received a combination of both local and systemic treatment, 4: Local intramammary infusion with Envirolyte-Anolyte 1:500, 5: Local intramammary infusion with AgNPs, 6: received both local treatment by intramammary infusion with 1:500 Envirolyte-Anolyte (2 mg of active chlorine) and received systemic treatment.

Figure 1: Different lines of treatment applied to cases of clinical mastitis and the cure percentages
The cured cases were 60% for the first group, 80% for the second and third one together with stimulation of the innate immune mechanisms of the animal. Improvement of local clinical signs of the swelling and milk appearance at the level of affected quarters by carprofen was observed, we think that carprofen may be able to relief most cases (80%), these observations are in accordance with Vangroenweghe et al., (2005).

The fourth and fifth group both were 60% while it was 100% in the sixth group which received both local treatment by intramammary infusion with 1:500 Enviroleyte-Anolyte (2 mg of active chlorine) and received systemic treatment by I/M injection of both enrofloxacin 5mg/kg body weight for 5 successive days and I/V injection of 2.9ml/kg body weight of carprofen.

The cure % of the first group may be due to insufficient to induce cure for this group.

Results of the study by Oliver et al., (2004) demonstrated that ceftiofur therapy was effecting in eliminating naturally occurring subclinical mastitis in lactating dairy cows when the therapy is extended more than 5 days.

Enviroleyte-Anolyte (1:500) and AgNPs were induced similar curing rate (60%) for 5 days local treatment.

The unique physiochemical properties of the nanoparticles combined with the growth inhibitory capacity against microbes has led to the upsurge in the research on nanoparticles and their potential application as antimicrobials. The mechanism of antibacterial effect of silver nanoparticles has been reported in the literature (Sondi and Salopek-Sondi, 2004), which suggests that the particles are bactericidal.

Several possible modes of action are discussed in the literature on nano-Ag effects on bacteria and fungi. These are (1) membrane disruption through direct attachment of the nanoparticle to the bacterial membrane, (2) cellular invasion and enzyme disruption by nanoparticles, (3) changes in cell membrane permeability (4) interference with cellular S-containing compounds, and (5) intracellular ROS accumulation (Hwang et al., 2008; Kim et al., 2009; Lok et al., 2006; Morones et al., 2005; Pal et al., 2007; Panáček et al., 2006; Sondi and Salopek-Sondi, 2004). That several of these events might act together to result in cell death is probable, but the specific processes and interactions required for toxicity have not been fully confirmed.

In one hand, in spite that, the third line was a combination of both second and third ones (intramammary infusion of 125 mg of ceftiofur hydrochloride plus systemic treatment by I/M injection of enrofloxacin and I/V injection of carprofen for 5 successive days), this line of treatment induced 80% curing of cases. These results are in accordance with those reported by Grewal et al., (2005). On the other hand it is interesting to say that, the combination of Enviroleyte-Anolyte (1:500) as intramammary infusion of mastitic udder and I/M injection of enrofloxacin and of carprofen for 5 successive days were induced 100% curing.

Conclusion

The unique physiochemical properties of the nanoparticles combined with the growth inhibitory capacity against microbes has led to the upsurge in the research on nanoparticles and their potential application as antimicrobials. Silver nanoparticles and superoxide activated water are representing new antimicrobial agents to develop the next generation of agents to control microbial infections in animal production.

References


