Preliminary study of homeopathic treatment of subclinical mastitis evaluated through somatic cells count and California mastitis test

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ABSTRACT

The present paper presents the results of homeopathic treatment of 25 Holstein breed cows aged 3 to 8 years old diagnosed with subclinical mastitis through California Mastitis Test (CMT). Animals were divided into 3 groups according with infection level. A homeopathic complex was developed on the grounds of clinical aspects, including Phosphorus 30x, Phytolacca 30x, Silicea 30x, Sulphur 30x, Belladonna 30x, Bryonia alba 30x, Pulsatilla 30x, Calendula 30x and biotherapeutic of *Staphylococcus aureus* 200x. The remedy was added to salt and was administered to cattle 100g/cow/day for 75 days. CMT were carried out every 2 weeks to control incidence and severity of mastitis; somatic cells count (SCC) was performed at the beginning and the end of treatment. CMT showed significant improvement in regression of infection level all throughout the study; final SCC showed decrease in 82% of animals, signaling thus efficacy of the homeopathic treatment.

Keywords: Subclinical mastitis; Dairy cows; Homeopathy; California Mastitis Test, Somatic Cells Count.

Introduction

Among the illnesses of most significant in cattle breeding, bovine mastitis is one among the ones causing the highest economic damage in the production of milk and dairy products due to reduction in production rate. Subclinical mastitis is responsible for 10% of the loss in cow/year productive capacity [1].

Mastitis is characterized as an inflammation of the mammary gland, usually of infectious etiology; it may be classified as clinical or subclinical. Clinical mastitis presents evident signs: udder swelling, high temperature, hardening and pain in the mammary gland, clots, pus or other alterations in milk [2]. Subclinical mastitis presents no macroscopic alterations, but there is alteration in the composition of milk [3]. It is the most significant form, as it is estimated that its relation to clinical mastitis is 9:1 [4].

Etiology of mastitis is complex and multiple, making the identification of pathogenic microorganisms essential for proper diagnosis, treatment and prevention. 140 agents have been identified, distributed among 35 classes; currently, *Staphylococcus aureus* is the most frequently isolated [5].

Detection of subclinical mastitis requires analysis of the cellular contents of milk. Increase on somatic cells (SC) is the main parameter used to diagnose subclinical mastitis. SC are classified as epithelial cells – arising from the normal peeling of the cover tissues or from internal secretor tissue of mammary glands, which corresponding to 2-25% of total SC in a sample. Around 75-98% of SC corresponding to the defense cells and includes leukocytes migrating from blood circulation to udders in infections. Therefore, the presence of SC may indicate an udder inflammation [6].

There are several tests to evaluate SC in milk, the use of California Mastitis Test (CMT) and Somatic Cells Count (SCC) is emphasized [7]. CMT is one of the most popular and practical tests and it is based on an estimation of SC in milk. Test results are rated as a function of the viscosity of a mixture of equal proportions of milk and reagent [7], milk is separately collected from udders into a tray where anionic detergent is added. The detergent breaks cell membranes with release of DNA, which presents high viscosity. According to intensity, reaction is classified as negative (zero), light (+), moderate (++) and intense (+++) [5].

Conventional treatment employs antibiotics and anti-inflammatory drugs. The use of these therapy results in economic loss for breeders due to high costs and milk discard from treated animals. A further disadvantage is the potential emergence of antimicrobial resistance [8].

For these reasons, homeopathy has been increasingly tested in animals, especially in cattle breeding [5], with positive results in several conditions, including mastitis [1,9,10]. The present study aimed at investigating the efficacy of homeopathic treatment as an alternative for subclinical mastitis in dairy herd.

Materials and methods

Study was carried out at a farm located in the district of Perobal, Paraná, Brazil, from June to August, 2007. 25 Holstein breed cows, 3 to 8 years old were divided into 3 groups named "blue", "yellow" and "black" respectively according to mammary infectious degree, +, ++ and +++, on the basis of CMT results. To carry out CMT, 2 mL of milk were collected from each mammary gland unit in a tray to which 2 ml of CMT reagent was added. SCC tests were also performed, following recommendations by *Program of Analysis of Dairy Cattle* of the State of Parana.

A homeopathic complex was prepared according to clinical data regarding: aspect of milk, aspect of udder, behavior of animals and microbiologic analysis of milk carried out at the Microbiology Laboratory of University Paranaense (UNIPAR), disclosing the presence of *Staphylococcus aureus*. On these grounds, the homeopathic complex included: Phosphorus 30x, Phytolacca 30x, Silicea 30x,

Sulphur 30x, Belladonna 30x, Bryonia 30x, Pulsatilla 30x, Calendula 30x and biotherapeutic of *Staphylococcus aureus* 200x Remedies were prepared following Brazilian Homeopathic Pharmacopeia [11]. Phytolacca, Belladonna, Bryonia, Pulsatilla and Calendula were preparing using mother tincture as base which was raised to 30x according Hahnemannian method for decimal dilutions. Silicea, Sulphur and Phosphorus, of mineral origin, were prepared by trituration and later dilution to 30x according to Hahnemannian method.

Biotherapeutic of *S. aureus* was prepared from freshly collected milk from 4 animals with the higher degree of infection on initial CMT, 1 mL of which was added to Tryptic Soy Broth (TSB) to promote bacterial growth cultured in greenhouse at 35°C for 24 hours. Then it was immediately replicated in a plate containing Purple Country Agar (PCA) to isolate the bacterial strain for identification; the dilution presented degree 3-standard-turbidity, measured through Mac Farland Scale. This dilution was used as the starting point for homeopathic manipulation, performed according Hahnemannian method in decimal scale.

After the homeopathic complex was prepared, it was added to mineral salt, an usual procedure in homeopathic practice, proportion was 10%. This was added to cattle feeding and administered in a dose 100g/cow/dose divided into 2 feeding periods, morning and afternoon, after milking the cows, for 75 days.

CMT was carried out every 2 weeks and SCC was performed at the beginning and the end of the study.

Results

After 15 days of treatment, CMT showed significant increase of mastitis. 2 weeks later, infection had decreased as well as the number of +++ degree udders. At 45 days, an increase of +++ degree udders was again seen, while at 60 and 75 days decrease was significant. (Figure 1)

Regarding SCC, decrease was observed in 82% of animals, among them 5 animals had decrease between 0-25%, 11 between 51-75%, 6 between 76-100% and 3 animals presented an increase. (Figure 2)

Figure 1: Results of CMT biweekly tests carried out during homeopathic treatment.

Figure 2: Decrease and increase in SCC, in percentage, after 75 days of treatment, in comparison to values before onset of treatment.

Discussion

The homeopathic complex was efficient as treatment for subclinical mastitis. This might be explained on several reasons. First, clinical examination was most significant as it allowed to choose the most suitable remedies for a complex composition representing the epidemic genus of the current infection [12].

Bryonia alba, Phytolacca decandra, Silicea terra and Belladonna corresponded to hardened, swollen, painful and congestive teats; Phosphorus was chosen due to the presence of fistulae in some teats; Pulsatilla was indicated for fistulous abscess and to increase the production of milk; Calendula, to promote healing of teats and maturation of abscesses.

Sulphur complemented the action of the other remedies and also the elimination of the infectious process [12-14]. The choice of dilution 30x for all these remedies was grounded on data described by Tiefenthaler [15], recommending the use of low dilutions (1-6X) for acute illness, organs and tissues; middle dilutions (8-12x) for subacute, functional disease and high dilutions 930x and higher) for chronic diseases and attending disturbs in behavior.

As in this case the main pathogenic agent was *Staphylococcus aureus*, agreeing with literature [4], biotherapeutic was prepared 200x according to the procedure described by Vervloet [16].

Significant increase in infection was observed after 15 days of treatment; this might correspond to homeopathic aggravation [6], probably due to the use of biotherapeutic *Staphylococcus aureus* 200x in the complex, causing transitional effects at the beginning of treatment.

On the other hand, according to Alves [6], SCC in infected animal joints is not stable, but suffers variations. In chronic infections, SCC as well as the number of bacteria tend oscillate in a remarkable way, increasing and decreasing along time. It can take days, weeks or even longer for SCC to decrease, and this only after pathogenic agents have been eradicated from the mammary gland.

Several factors may influence SCC, including individual and environmental. This helps to explain SCC increase in 3 animals as shown in Figure 2.

A further observation in this study showed that the handling of animals during milking can significantly influence response to remedy. For this reason it is important to establish a milking routine.

Cows with infections, mainly mastitis, should be milked after the healthy animals in order to avoid contamination of the former. It is recommended to milk animals in groups according to health conditions, in the following order: first, primipare heifers, then cows who have never had mastitis followed by the healed ones and finally, animals under treatment [17].

Conclusion

Through CMT and SCC tests, it was observed that the animals treated with a homeopathic complex presented a decrease in SC values. This was due to special care into individualizing the current epidemic genus of mastitis and to the use of a milking line system.

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