

THERAPEUTIC EVALUATION OF AUTOGENOUS MASTITIS VACCINE ALONE AND IN COMBINATION WITH ROUTINE THERAPY IN SUBCLINICAL MASTITIS

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Mastitis is one of the most economically important diseases of dairy animals in Pakistan. The cure rate of mastitis with the use of antibiotics/antibacterials leaves a lot to be desired. In addition, the use of these agents leads to undesirable residues in milk and poses a host of problems to milk processing industry. Therefore, measures other than the use of antibiotics/antibacterials are being investigated to improve the mastitis cure rate. Keeping these considerations in perspective, the current study was planned to evaluate the therapeutic efficacy of autogenous animal-specific mastitis vaccines alone and in combination with antibiotic based rational therapy. For this purpose, preparation of autogenous mastitis vaccine was carried out from the isolates of individual animals suffering from subclinical mastitis (n=60). The selected animals were randomly divided into 3 equal groups. Group A received autogenous mastitis vaccine alone, group B received routine mastitis therapy while group C received combination of both these treatments. The efficacy of the treatments was evaluated through Surf Field Mastitis Test (SFMT) based cure rate, bacteriological cure rate and somatic cell counts (SCC). The treatments led to an SFMT based cure rate of 75%, 80% and 85% respectively. The bacteriological cure rate observed for groups A, B and C were 70%, 70% and 75% respectively, at completion of study period, whereas there was statistically non-significant difference in SCC values in all the groups. The study reveals that autogenous mastitis vaccine led to a comparable results and can be a suitable replacement for antibiotic therapy in subclinical mastitis without any fear of antibiotic residues.

Keywords: Livestock, dairy animals, bacteria, milk infection, mammary gland, antibiotic therapy, autogenous vaccine

INTRODUCTION

Among various threats faced by dairy animals like HS, FMD and Ketosis mastitis is one of the major livestock problems in Pakistan (Bachaya *et al.*, 2011). Financial damages (438 kg milk lost and 57 days shorter lactation period in affected buffaloes) caused by mastitis are not only in terms of cost involved for antibiotics and cost incurred on management practices but it also includes milk loss, premature culling, mortality as well as infected milk discarding (Yadva, 2018). Mastitis is an aftermath of interaction between host, agent and environment (Radostits *et al.*, 2007). It has a multifaceted etiology and a wide range of microorganisms have been associated with this menace in dairy animals. Most frequently, mastitis is caused by *Staphylococcus aureus*, *E. coli*, *Streptococcus agalactiae*, *Corynebacterium pyogenes* (current designation= *Trueperella pyogenes*), *Pseudomonas aeruginosa* and yeast cells. In Pakistan, *Staphylococcus aureus* and *Streptococcus agalactiae* are reportedly the most prevalent causative agents of mastitis in dairy animals particularly in cattle and buffaloes (Shakoor, 2006; Jones *et al.*, 2009). Due to multifactorial etiology, mastitis control and its treatment is often challenging (Tenhagen *et al.*, 2006; Deb *et al.*, 2013).

For treating mastitis in dairy animals, antibiotic therapy is the common practice. However, it has become so challenging to prevent and treat the mastitis by using antibiotics. Undesirably low cure rate, increased cost of treatment, antibiotic resistance and public health concern regarding residues in milk and meat have enhanced the interest of researchers to treat and prevent mastitis by using vaccine instead of antibiotics (Abera *et al.*, 2013). Vaccinating the cows during lactation may be considered beneficial to enhance the resistance of cow against infection of the mammary gland. Mastitis vaccination is a technique of administering attenuated or killed pathogen in the body or in the udder of a cow which make the animal protected against that specific organism or toxins. As a result of this, a robust antibody titer is desirable to develop in animal's body against that specific organism.

An autogenous vaccine is defined as "a vaccine that is prepared from a microorganism (s) isolated from a sick animal, which the attending veterinarian believes, is the causative agent (s) of the disease affecting the herd". These vaccines are useful for the treatment of several different infections, including those caused by mastitis pathogens which are intracellular like *S. aureus*. Autogenous vaccines are a good option when immunity to an organism is strain

specific (Czernomysy-Furowicz *et al.*, 2014). Various efforts have been put forth into use of autogenous vaccines as alternatives or adjuncts to antibiotics. Keeping the pitfalls of conventional antibiotic mastitis therapy and potential of autogenous mastitis vaccines in perspective, the present study was designed to evaluate the therapeutic efficacy of autogenous mastitis vaccines alone and in combination with antibiotic based rational therapy, and to determine the cost-effectiveness of autogenous vaccine alone and in combination with rational therapy.

MATERIALS AND METHODS

A total of 60 sub-clinically mastitic cows and buffaloes (screening through SFMT) belonging to small holder farmers and commercial or institutional farms were selected for the evaluation of animal-specific autogenous mastitis vaccine alone and in combination with standard mastitis therapy.

Isolation and identification of bacteria: The affected quarters were subjected to microbiological examination of milk for determination of mastitis pathogens (Hogan *et al.*, 1999).

Preparation of autogenous mastitis vaccine: Inoculation of vaccinal isolates on to blood agar was carried out and these were incubated at 37°C for 24 h. After this, washing of the bacterial growth from the surface of the agar was performed with a sterile normal saline solution. Then transfer of bacterial suspension into a new sterile tube was carried out in a volume of 10 mL which was standardized to 1 McFarland standard (3×10^8 CFU/mL) and inactivation was performed by adding 0.05mL of formalin. Aluminum hydroxide gel was used as adjuvant (Sears, 2002). The sterility of the prepared autogenous vaccine was confirmed by its inoculation (0.5mL) on to Columbia blood agar followed by incubation at 37°C for 72 h. The absence of a colony was interpreted as lack of viable bacteria in the prepared autovaccine.

Administration of the vaccines: Selected mastitic cows and buffaloes were randomly assigned to the following three treatments.

Group 1: Autogenous mastitis vaccine @ 5ml/animal I/M twice at an interval of two weeks.

Group 2: Customary mastitis therapy i.e. Tylosin (10mg/kg) for three days I/M + amoxicillin & clavulanic acid (600mg) for 5 days daily, I/mm infusion (Ahmad, 2009)

Group 3: Combination of autogenous vaccine and standard mastitis therapy (Czernomysy-Furowicz *et al.*, 2014; Ahmad, 2009).

Evaluation criteria: The efficacy of the above three treatment protocols was evaluated on day 0, 14 and 28 post treatment on the basis of SFMT based cure rate (Muhammad *et al.*, 2010), Bacteriological cure rate (Hogan *et al.*, 1999) and Somatic cell count (SCC) (Athar, 2007).

RESULTS

Effect of animal specific autogenous mastitis vaccine alone and in combination with antibiotic therapy on surf field mastitis test (SFMT) based cure rate in subclinically mastitic animals: The values of SFMT based cure rate observed for animals suffering from subclinical mastitis are given in Table 1. Out of 20 selected animals of group A, administration of the allocated treatment (autogenous mastitis vaccine) resulted in a 65% SFMT based cure rate at day 14 post initiation of the treatment which further improved to 75% at the end of study period. Administration of rational mastitis therapy (antibiotics) in animals (n=20) of group B led to a 60% SFMT based cure rate at day 14 after start of therapy. At the end of trial, SFMT based cure rate observed in the group was 80%. The combination of treatments (autogenous mastitis vaccine and rational therapy) in subclinically mastitic animals of group C led to 65% SFMT based cure rate at day 14 after start of therapy which improved to 85% at day 28 after start of therapy.

Table 1. Effect of animal specific autogenous mastitis vaccine alone and in combination with antibiotic therapy on surf field mastitis test (SFMT) based cure rate in subclinically mastitic animals.

Group	Total SFMT +ve Animals	SFMT based Cured on day 14 post treatment	SFMT based Cured on day 28 post treatment
A	20	13 (65%)	15 (75%)
B	20	12 (60%)	16 (80%)
C	20	13 (65%)	17 (85%)

Group A (n=20) = Administration of autogenous mastitis vaccine 5ml/animal (IM) twice seven days apart. Group B (n=20) = Administration of tylosin (10mg/kg) for three days + amoxicillin and clavulanic acid (600mg) I/mm infusion daily for 5 days. Group C (n=20) = Administration of autogenous mastitis vaccine 5ml/animal (IM) twice seven days apart + administration of tylosin (10mg/kg) for three days + amoxicillin and clavulanic acid (600mg) I/mm infusion daily for 5 days.

Effect of animal specific autogenous mastitis vaccine alone and in combination with antibiotic therapy on bacteriological cure rate in subclinically mastitic animals: The values of bacteriological cure rate observed for animals suffering from subclinical mastitis are given in Table 2. In 20 selected animals of group A, administration of the allocated treatment (autogenous mastitis vaccine) resulted in a 55% bacteriological cure rate at day 14 post initiation of the treatment which further improved to 70% at the end of study period. Administration of rational mastitis therapy (antibiotics) in animals (n=20) of group B led to a 60% bacteriological based cure rate at day 14 after initiation of treatment. A 70% bacteriological cure rate was recorded in the group at day 28 after start of therapy as milk samples collected yielded no bacterial growth. The combination of

treatments (autogenous mastitis vaccine and rational therapy) in mastitic animals of group C led to 60% and 75% bacteriological cure rate at day 14 and 28 after start of therapy, respectively.

Table 2. Effect of animal specific autogenous mastitis vaccine alone and in combination with antibiotic therapy on bacteriological cure rate in subclinically mastitic animals.

Groups	Total microbiologically positive animals at day 0 (baseline)	Cured animals out of total at day 14	Cured animals out of total at day 28
A (n=20)	20	11 (55%)	14 (70%)
B (n=20)	20	12 (60%)	14 (70%)
C (n=20)	20	12 (60%)	15 (75%)

Group A (n=20) = Administration of autogenous mastitis vaccine 5ml/animal (IM) twice seven days apart. Group B (n=20) = Administration of tylosin (10mg/kg) for three days + amoxicillin and clavulanic acid (600mg) I/mm infusion daily for 5 days. Group C (n=20) = Administration of autogenous mastitis vaccine 5ml/animal (IM) twice seven days apart + administration of tylosin (10mg/kg) for three days + amoxicillin and clavulanic acid (600mg) I/mm infusion daily for 5 days.

Effect of animal specific autogenous mastitis vaccine alone and in combination with antibiotic therapy on somatic cell count in subclinically mastitic animals: The values of Somatic Cell Count (SCC) observed for animals suffering from subclinical mastitis are given in Table 3.

Table 3. Effect of animal specific autogenous mastitis vaccine alone and in combination with antibiotic therapy on somatic cell count in subclinically mastitic animals.

Groups	Days after start of therapy		
	Baseline	14	28
A (n=20)	8.34±0.32	3.05±0.29	2.19±0.22
B (n=20)	8.04±0.33	3.30±0.44	2.26±0.25
C (n=20)	8.32±0.31	2.97±0.31	2.39±0.24

Group A (n=20) = Administration of autogenous mastitis vaccine 5ml/animal (IM) twice seven days apart. Group B (n=20) = Administration of tylosin (10mg/kg) for three days + amoxicillin and clavulanic acid (600mg) I/mm infusion daily for 5 days. Group C (n=20) = Administration of autogenous mastitis vaccine 5ml/animal (IM) twice seven days apart + administration of tylosin (10mg/kg) for three days + amoxicillin and clavulanic acid (600mg) I/mm infusion daily for 5 days.

These values indicate a statistically significant ($p<0.05$) elevation in milk SCC values than normal in subclinically mastitic animals. Provision of the allocated treatments resulted in a statistically significant ($p<0.05$) decrease in SCC values in all the treatment groups at 14th day after initiation of treatment comparing to baseline. A similar trend was

observed at next sampling time point i.e. day 28 after start of therapy. A statistically non-significant ($p>0.05$) difference was observed among the three treatment groups at the end of study period in terms of SCC values.

DISCUSSION

Mastitis is one of the leading causes of huge economic losses to dairy industry. The treatment of mastitis mainly relies on use of antibiotics parentally as well as locally. But this antibiotic usage brings many harms along with itself like low cure rate, antimicrobial resistance and antibiotic residues (Sol *et al.*, 2000; Sears and McCarthy, 2003). The latter two are of prime importance keeping in view “one health perspective”. So alternatives to antibiotic in devising mastitis therapy are dire need of time which should preferably be non-antibiotics. Effective vaccine can step into shoes to fill this dire need effectively. But multifaceted etiology of mastitis creates a big hurdle in effective cure and prevention of the disease by using vaccination. To circumvent this issue autogenous mastitis vaccine can be used; as it will be comprising of the etiological agents yielded at the specific farm or area.

The SFMT based cure rate in subclinically mastitic animals accounted for 65, 60 and 65% for groups A, B and C respectively, at day 14 after start of therapy. The SFMT based cure rate observed at the end of study period for subclinically mastitic animals was 75, 80 and 85% for groups A, B and C, respectively. These findings are in broad agreement with those reported by Czernomysy-Furowicz *et al.* (2014) as better recovery from clinical severity and reoccurrence of clinical mastitis was found when autogenous mastitis vaccine was used along with antibiotics in mastitic animals. Similarly, Piepers *et al.* (2016) found that there was less incidence of induced mastitis in experimentally infected mastitis when the cows were vaccinated with autogenous mastitis vaccine.

The administration of animal-specific autogenous mastitis vaccine to subclinically mastitic animals of group A led to 55% (11 out of 20 animals) bacteriological cure rate whereas administration of rational mastitis therapy to animals of group B resulted in 60% (12 out of 20 animals) at day 14 after start of therapy. The combination of both the treatments when administered to animals of group C caused bacteriological clearance of milk samples in 12 (60%) animals. A 70, 70 and 75% bacteriological cure rate was observed at the end of study period (day 28 after start of therapy), respectively. These findings correspond the findings of Czernomysy-Furowicz *et al.* (2014) who have reported a better infection clearance in autogenous vaccine treated mastitic animals. Similarly, in separate studies, Prenafeta *et al.* (2010), Leitner *et al.* (2003) and Hwang *et al.* (1999) also found a better bacteriological cure rate when mastitic animals were treated with *Staphylococcus aureus* vaccine compared with antibiotics.

Animals suffering from subclinical mastitis had a rise in normal range of Somatic Cell Count (SCC) and

administration of animal-specific autogenous mastitis vaccine alone to animals of group A, routine antibiotic mastitis therapy to animals of group B and combination of protocol I and protocol II to the animals of group C resulted in a statistically significant decrease ($p < 0.05$) in SCC values at day 14 after start of therapy. A further statistically significant ($p < 0.05$) decrease in SCC values of all the groups was observed at day 28 after start of therapy (last sampling time point). The difference between the treatments was non-significant ($p > 0.05$). Comparable outcomes have been described by Leitner *et al.* (2003) where a significant reduction in SCC values was evident after provision of *Staphylococcus aureus* vaccine as a treatment of mastitis in a field trial.

The major mastitis pathogens are intracellular in nature. It has been proven that during the infection of the udder and resultant inflammation, mostly IgG2 antibodies are produced by supra mammary lymph nodes and are secreted into the udder. On the other hand, it has also been proven that IgG2 are not sufficient enough to combat intracellular pathogens because antigen presentation is not possible in such cases. Administration of autogenous vaccine leads to antigen presentation through antigen presenting cells to T helper 1 (Th1) lymphocytes, due to which B cells are stimulated and specific IgG2 antibodies are produced by production of Interferons (IFN- γ). Revaccination (booster dose) leads to quicker reproduction of antibodies which remain for longer duration. Moreover, autovaccines are also considered to enhance the phagocytic activity of neutrophils which leads to better infection clearance.

Conclusion: The findings of the trial suggest that autogenous mastitis vaccine alone or even in combination to antibiotic therapy can become a part of armamentarium to fight this global issue of mastitis. The technique has not only comparable results to rational antibiotic therapy but also there is no danger of milk residues, emerging antibiotic resistance which results in creation of super bugs and discarding of milk.

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