Mastitis is a multi etiological complex disease, which is defined as an inflammation of parenchyma of mammary glands. It is characterized by physical, thermal, chemical, mechanical and usually bacteriological changes in milk and pathological changes in glandular tissues. It is the most common and most costly disease of dairy cattle. Mastitis continues to be a cause of significant economic loss to the dairy industry. Despite significant advances, mastitis remains a problem in dairy herds. It impacts on animal health and welfare, on market image, on profitability and, critically, on the quality of life of dairy farmers. More than 250 different microorganisms can cause mastitis. Even no single vaccine is successful to control mastitis due to its multi etiological nature. However, antibiotics were introduced 50 years back for its control. But the problem in dairy animals remained as it was prior to antibiotic era. Dairy production is a biologically efficient system that converts feed and roughages of milk. Milk is very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals.

Mastitis causes heavy economic losses to the dairy industry worldwide. The first report on mastitis caused losses in India was about Rs.529 crores annually. These losses increased to Rs.6053.21 crores in the year 2001. Nearly 70% of this loss is a result of reduced milk production caused by sub-clinical mastitis. Apart from its economic importance it is also a matter of concern of carries public health significance. Moreover, presence of antibiotic residues in the milk is undesirable due to its public health concern. Traditionally, the mastitis control programmes are focused at use of chemical disinfectants, antiseptic or herbal teat dips and antibiotic therapy. In herds without an effective mastitis control program, about 40% of the cows are infected in an average of two quarters. Reduced milk production accounts for about 70% of the total loss associated with mastitis. The inflammatory response consists of an increase in blood proteins and white blood cells in the mammary tissue and the milk.
Surveys of the prevalence of mastitis in most countries, irrespective of the cause, show a comparable figure of 50% among dairy cows and a quarter infection rate of 25% (Radostits et al., 2000). Subclinical mastitis is believed to be more prevalent than clinical mastitis in most countries. Mukharjee (2009) have been reported the overall prevalence of clinical mastitis and subclinical mastitis were 15.18% and 42.93% respectively during the month of July and August in Uttar Pradesh. Sharma (2009) had been reported prevalence of subclinical and clinical mastitis 42.18% and 10.93% respectively in dairy cows in Jammu. A study in Jammu by Sudhan et al. (2005) suggests that Staphylococcus aureus (56.89%) is major pathogen followed by Micrococcus spp. (15.51%), Bacillus cereus (12.06%), Staphylococcus epidermidis (8.62%), Klebsiella spp. (3.44%), Escherichia coli (1.72%), and Corynebacterium spp. (1.72%). Sharma et al. (2007) also isolated Staphylococcus aureus and Escherichia coli from acute clinical mastitis in buffalo.

The antibiotic treatment may be help but in minimizing the losses but simultaneously may lead drug resistance. Factors such as pharmacokinetic problems and phagocytosis depressing effect of certain antibiotic and appearance of residue in milk restrict the success of antibiotic therapy.

When an antibiotic treatment is recommended, it is very critical to follow instruction, especially regarding the duration of treatment. Only mastitis caused by S. agalactiae can be treated successfully with antibiotics during lactation. The success rate of antibiotics in treating mastitis cause by S. aureus and coliform bacteria does not exceed 50% could be as low as 10%. Additional losses are associated with changes in milk quality and composition culling. The possibilities of drug residues in milk also increased.

Some alternatives have been developed, which include caprylic acid and monocaprylin, lysostaphin, oxytocin and vaccines. Presently, vaccination is one of the most widely accepted methods for treating mastitis. Several vaccines are commercially available, such as
LYSIGUIN (a vaccine against *Staphylococcus aureus* mastitis) and *E. coli* O111:B4 mutant strain J5 (a vaccine against *E. coli* mastitis). However, almost all vaccines used presently are active vaccines, which are utilized mainly for immunoprophylaxis. Therefore, attention is being paid to find alternative approaches. Antibiotics are used widely in prevention and treatment of dairy cow mastitis with the present awareness of the problems associated with antibiotics resistant and its residue, its urgent to find an alternative to antibiotics, chicken egg yolk immunoglobulin(IgY) provides an inexpensive and effective source of antibodies for the passive immunization of animals. Its promoting alternative for the treatment and prevention of bacteria infections, and has shown to be effective against a number of pathogens.

Specific egg yolk immunoglobulin (IgY) can be produced in egg yolk by immunizing hens with specific antigens. The IgY can then be isolated in large quantities from yolk by simple methods without distress to the birds.

Therefore, IgY as a passive, inexpensive and easy producing vaccine has attracted much attention and been recognized to be efficient in therapy and prevention. It has reported oral administration of polyclonal IgY against *Streptococcus agalactiae* and *S. aureus* was efficacious for lowering somatic cell counts (SCCs) in dairy cows. IgY technology fulfills this requirement, since chicken antibodies can be easily sampled by a noninvasive method based on the simple action of the egg collection, instead of the stressful bleeding of animals to obtain serum. IgY technology also offers outstanding economical advantages because the costs for hen keeping are lower than those for rabbits. Furthermore Ab production of hen roughly corresponds, to that of a large mammal, such as a sheep or a goat. Thus an extraordinary amount of Ab can be produced from only one hen, approximately 17-35g of total IgY. Powdered whole eggs or yolks have been used in veterinary medicine as an inexpensive IgY source for the treatment of enteric diseases.
The yolk of eggs laid by immunized chickens has been widely recognized as an excellent source of polyclonal antibodies (pAb) for over a decade. Specific antibodies produced in chickens offer several important advantages over producing antibodies in mammals. Due to the phylogenetic distance between birds and mammals of specific antibody against mammalian antigens when using chickens. Highly conserved mammalian proteins sometimes fail to elicit a humoral immune response in animals, such as rabbits, that are traditionally used for generating pAb. Since chicken IgY does not cross-react with mammalian IgG and does not bind bacterial or mammalian Fc receptors, and the need for cross-species immunoabsorptions also is eliminated. Moreover, IgY purification does not require animal bleeding. A single egg contains as much antibody as an average bleed from a rabbit, thus this simple, non-invasive approach presents an appealing alternative to conventional pAb production methods.

Hence the present investigation focused attention in generation of antibodies against *Escherichia coli* and *Staphylococcus aureus* separately, which could be used for the treatment of two different conditions at single attempt by mixing together as consortium in the future progression of this study.

**The objectives of the present study are as follows,**

- Isolation of *Escherichia coli* and *Staphylococcus aureus* from mastitis infected milk sample.
- To prepare and standardize the whole cell antigens of *Escherichia coli* and *Staphylococcus aureus*.
- To generate antibodies against the prepared antigens in 21-weeks old white leghorn chickens.
- To purify and characterize anti-*Escherichia coli* antibodies and anti-*Staphylococcus aureus* antibodies from the egg yolk of immunized chickens.
- To evaluate the specificity of the purified chicken IgY.
• To determine the physiochemical properties and stability of egg yolk antibodies.
• To measure the inhibitory activity of the IgY against the pathogens using growth inhibition assay.
• To prepare spray dried and freeze dried egg yolk powder from the eggs of the immunized hens.