Bovine mastitis is an inflammation of the mammary gland. The two major bacterial pathogen, *Staphylococcus aureus* and *Escherichia coli* leads to considerable economic losses for the dairy industry (Gill et al., 2006). Many micro organisms cause mastitis, and these vary greatly in the route by which they reach the cow and in the nature of the disease. At present antibiotics such as penicillin, methicillin, erythromycin etc., are primarily used for the therapy of mastitis. Milk and milk products from the infected cow contain toxins secreted by the microbes and antibiotic residue which may lead to severe health hazards to humans. The other demerit associated with the antibiotic therapy is the occurrence of multiple serotypes of organisms inducing the infection, so the vaccine are not very specific for the treatment of disease. Furthermore, the increasing prevalence of antibacterial-resistant bacteria has reduced the effectiveness of antibacterial therapy (Guler et al., 2005).

Recently, the vaccination via antibodies produced by the vaccinated animal is followed and it acts as another alternative which might be more attractive. This antibody therapy is of great importance (Coleman, 1996). The mammalian antibodies IgG and the chicken egg yolk antibodies came into play a major role in the diagnosis of diseases in poultry and dairy industry. Traditionally, the rabbit antibodies (IgG) were used for this purpose. But laboratory production of antibodies involves immunization and bleeding of animals, causing distress to them. Egg yolk immunoglobulin can be isolated from the egg yolks of immunized hens by several simple steps without distressing the birds (Akita and Nakai, 1993). IgY can be easily produced and this antibody has received much attention and was found to efficiently prevent or control pathogen infections in animals (Carlander et al., 2002). The European Centre for the Validation of Alternative Methods (ECVAM) recommends that Egg yolk
immunoglobulins should be used instead of mammalian antibodies for animal welfare reasons (Carlender et al., 2002).

Specific IgY can prevent or control infections caused by *E. coli* in piglets (Jin et al., 1998), by *rotavirus* in calves (Kuroki et al., 1994), and by *Salmonella* in mice (Gurtler et al., 2004). Coleman and Marilyn (1996) reported oral administration of polyclonal IgY against *Streptococcus agalactiae* and *S. aureus* was efficacious for lowering somatic cell counts (SCCs) in dairy cows, but there is no report so far on IgY controlling mastitis caused by *E. coli*.

Chickens store high contents of IgY in the yolk and are considered to be efficient antibody producers (Gottstein and Hemmeler, 1985). In a period of 6 wk, one immunized hen produces 298 g of IgY, which is much higher than the serum antibody (16.6 mg) obtained from one rabbit. Moreover, due to the phylogenetic distance between birds and mammals, chickens produce more specific antibodies against mammalian antigens than do mammals. The IgY is superior to serum antibody due to higher levels of specific antibodies and relative ease of purification (Akita and Nakai, 1992) with low cost (Polson and Von Wechmar, 1980). The egg yolk antibody has also an advantage over the serum antibody because of its compatibility with modern animal protection regulations (Gottstein and Hemmeler, 1985).

In this present study the biochemical characteristics of the standard culture causing bovine mastitis was studied. On the basis of the advantages of IgY over the mammalian antibodies entitled in the previous report, the present study focused to develop egg yolk antibodies to control the morbidity and mortality of the dairy industry from the infection and diseases caused by the predominant bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus*, instead of the treating the infected cattles using antibiotics. The prepared whole cell antigens were used to immunize the 21 weeks old
white leghorn chickens to generate IgY. Subsequent booster doses were given at weekly interval to raise the antibody titre in the egg yolk. The eggs were collected, stored and antibodies were purified from chicken egg yolk by Polson et al., (1980) method. The molecular weight of the purified IgY’s were confirmed as 180KDa through SDS PAGE (Laemmli, 1970). The electrophoretic band pattern obtained in this study was similar to that of the bands obtained by Kariyauasam et al., (2004).

The protein concentration of the egg yolk from the eggs of the 21 weeks old immunized chicken was found to be 4.5 mg/ml. This concentration of protein increased slowly and reached a steady level giving a maximum content of 5.1 mg/ml and 5.3 mg/ml for anti- *Staphylococcus aureus* IgY and anti-*Escherichia coli* IgY respectively at the 160th day after immunization. Similarly, the total IgY concentration of egg yolk was ~6.2mg/ml of egg yolk during the 7th day of immunization after which there was a steady increase in the concentration to reach a maximum of 6.8 mg/ml and 7.2 mg/ml for anti-*Staphylococcus aureus* IgY and anti-*Escherichia coli* IgY respectively. Moreover, the results showed that irrespective of the antigen used the amount of total IgY in the egg yolk had no considerable variation. This was in association with the findings reported by Lee et al., 2002, which indicated the total IgY concentration was independent of the type of antigens used to generate antibodies.

The specific antibody level in chicken serum and the titre of Anti-*E.coli*-IgY and Anti-*S.aureus*-IgY in dilution of IgY-extracts obtained from the eggs of laying hens immunized with antigens were determined by Indirect ELISA using the antigen and mentioned as optical density (OD) at 405nm (ELISA value). An increase in antibodies against respective antigens was detected in the serum of immunized chicken 7days after the initial immunization by ELISA. This humoral
immunity reached a peak at about 45 days. However, in the egg yolk, the antibody level increased 2 weeks after the initial immunization and persisted increase was observed till the 56th day after which the antibody level remained stable till the 160th day of immunization. The titre of specific antibody was found to be 1:10000 on 56th Day and the titre were maintained with booster doses. These results were comparable to the work done by O’Farrelly et al., 1992, which showed that antibodies started to appear in serum 10 days after immunization began and reached high titre at 45th day and remained stable till 168th day observation. This long lasting titre of antibodies correlated with the results of Losch et al., 1986.

In order to evaluate the efficacy of IgY in the prevention and treatment of E.coli and S.aureus infection in chickens, the stability of IgY was investigated at different physicochemical conditions. The specific reactivity of IgY when incubated as purified IgY or as liquid yolk at different temperature, different pH, pepsin and trypsin was assessed by measuring the residual activity using ELISA after each treatment. The results showed that the purified IgY was stable at 4°C, 10°C, 25°C and 37°C. Approximately 25% of its activity was lost at 60°C and then significantly decreased at 70°C. It was almost completely lost at 80°C. The purified IgY was stable between pH 4.0 and pH 10.0, it has retained only 20% of its activity at pH 2 and completely lost its activity at pH 12.0. In liquid yolk, IgY was relatively stable to high temperature at 80°C and complete loss of activity at 90°C. The pH stability of IgY with liquid yolk was found to be pH 3.0 to 11. The activity of purified IgY after pepsin treatment was almost completely lost but 40% of IgY activity was recovered when it was in liquid yolk. In contrast to pepsin treatment, purified IgY showed broad stability to trypsin, approximately 80% of the antibody activity was still remained. Tyrpsin did not seem to have any detrimental effect on IgY when it was in liquid yolk. These results revealed that IgY was
relatively stable to high temperature and broad pH range in the presence of its natural form (egg yolk) and IgY was more resistant to the effects of trypsin compared to pepsin. These observations were indicating that once the IgY passes the acidity of the stomach, it could retain most of its activity and therefore, can combat or minimize the effect of intestinal pathogens such as *Escherichia coli* and *Staphylococcus aureus* when it is given as natural form (egg yolk). Similar results were reported by Jaradat and Marquardt (2000).

Growth inhibition assay was performed to investigate that the binding activity of Anti-*Escherichia coli*-IgY and Anti-*Staphylococcus aureus*-IgY could inhibit the respective organisms growth in the liquid medium. The growth of *Escherichia coli* showed a lag phase of 0 to 2 hours, exponential phase of 2 to 6 hours and then stationary phase after 6 hours of incubation at standard conditions. With the similar conditions, the growth of *E. coli* with their respective specific IgY and non-specific IgY was plotted for growth inhibitory assay. In this assay the IgY concentration i.e., 360mg/ml was used on the basis of previous study done by Lee et al., (2002). The growth of *Escherichia coli* and *Staphylococcus aureus* with specific IgY showed significant reduction after 4-6 hours of incubation when compared to the growth with non-specific IgY. The results were comparable to the report of Lee et al., (2002). As a result it was observed that both the specific IgY of respective cultures were found to inhibit the growth of homologous cells in a liquid medium. The mechanism of action by which the antibodies suppress the growth was not clearly understood, but there are some proposed reasons discussed by Lee et al., (2002); Sunwoo et al., (2002) and Mahadavi et al., (2010). The binding of specific IgY to bacterial surface components could cause some structural alterations of the bacterial surface, which may block the opportunity to take nutrients and proliferate. This could be because antibodies were generated against whole bacterial cell, which could possess binding
activities against various epitopes of the bacterial surface as is the characteristics of a polyclonal antibody. Therefore, binding activities of IgY against bacterial surface components, including fimbriae and outer membrane protein may cause the growth inhibition. The results revealed that the specific binding activity of IgY is an important factor for the major antibacterial property. However, it is essential to conduct more intensive studies to explore the exact mechanism of inhibition.

In conclusion, specific IgY antibodies was produced by immunizing hens with a mastitis associated *Staphylococcus aureus* and *E.coli*. IgY as a complement or alternative to antibiotics offers a possibility to avoid development of antibiotic resistance. Passive immunotherapy with specific IgY may be a promising alternative with high specific nature and low cost effective.