The present investigation was aimed to generate chicken egg yolk antibodies against antigens of *Staphylococcus aureus* and *Escherichia coli* to test their potential *in vitro* to control the mastitis which cause major loss in dairy industry.

The antigens required for the generation of antibodies in the chicken were prepared from the standard strains of *Staphylococcus aureus* and *Escherichia coli*. The 21 weeks old white leghorn chicken were immunized individually for each antigen, their eggs collected and the antibodies were purified. An increase in antibodies against *Staphylococcus aureus* and *Escherichia coli* was detected in the serum of immunized chicken 7 days 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 and the titre were maintained with booster doses.

The protein concentration of the immunized chicken egg yolk 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 ~6.2 mg/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.

Purity of IgY was determined by SDS-PAGE, in which, 180KD protein bands were observed. The stability of IgY when incubated at different conditions was assessed where the IgY retained its stability at 4°C, 25°C, 37°C and 60 °C, at pH ranges between 4
and 9, where the IgY did not reduce its activity, when compared to the untreated control.

Titer of specific IgY in egg yolk was estimated by ELISA and MAT. A peak titer of more than 1:100000 were observed on 35th day onwards. In MAT the peak titer was 1:2560 on 49th day onwards.

The specific activity of IgY to the bacterial antigens were determined by Growth inhibition assay, the growth rate in the presence of IgY fractions was decreased due to the binding of specific IgY to the bacterial surface components and structural alterations of the bacterial surface have been suggested as the mode of action of specific IgY to inhibit bacterial growth.

The results of this study provides a platform for the control of mastitis using antibodies prepared from chicken egg yolk (IgY) against the mastitis causing bacteria *Staphylococcus aureus* and *Escherichia coli*. Thus the chicken egg yolk anti-*Escherichia coli* antibodies and anti-*Staphylococcus aureus* antibodies can be used for the both diagnosis and prevention of Bovine mastitis.