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DEVELOPMENT OF AN ELISA BASED DETECTION KIT USING CHICKEN EGG YOLK ANTIBODY AGAINST GASTROINTESTINAL INFECTION CAUSING *Salmonella enteritidis* IN POULTRY

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Abstract: *Salmonella enteritidis* is a serotype of *Salmonella enterica* which causes Non-typhoidal and Gastro-intestinal infection in both humans and animals. Chicken IgY has been identified as an alternative for the treatment of non-typhoidal salmonellosis. 21 weeks old white leghorn chicken which lays eggs was immunized with *Salmonella enteritidis.* The anti-Salmonella enteritidis IgY was purified and their protein content was estimated. The specificity of produced IgY was tested with Rapid Agglutination test, Micro agglutination test and Cross reactivity test. SDS-PAGE analysis of IgY showed a protein profile of the IgY, antibody titer potency of each serum and IgY was determined by Indirect ELISA. The present study was conducted to develop both Direct and Indirect ELISA kit for the detection of *Salmonella enteritidis* infection.

Keywords*:* Gastro-intestinal infection, *Salmonella enteritidis*, IgY, ELISA, SDS-PAGE, Micro agglutination, Cross reactivity, ELISA kit

I.INTRODUCTION

GIT infections are viral, bacterial or parasitic infections that cause gastroenteritis, an inflammation of the GI tract involving both in the stomach and the small intestine. Microbiology of bacterial gastrointestinal infections which are mainly caused by *E. coli, Salmonella, Shigella, Campylobacter, Clostridium*. The Gastrointestinal infection include Nausea, vomiting, fever, Dehydration, blood in the stool. Among these bacterial species *Salmonella* plays a major role in causing many gastrointestinal infections. *Salmonella* infection (salmonellosis) is the most common bacterial disease that affects the intestinal tract of human and animals. *Salmonella* bacteria live in human and animal intestines and they are spread through feces. It is the infection of the digestive tract by the bacterium *Salmonella enteritidis. Salmonella* is a widespread organism and it can be found on many dairy farms and also in many species of animals, birds, insects and reptiles. *Salmonella enteritidis* is a rod shaped, gram negative, non-motile bacteria. That does not form spores. Unlike other strains of *Salmonella* that are primarily adopted to people, *Salmonella enteritidis* is primarily adopted to animal host at least for the beginning of its life cycle. *Salmonella enteritidis* are considered facultative anaerobes, which means that these bacteria can survive with or without oxygen. *Salmonella enteritidis* move through their host intestine via flagella. Production of IgY antibodies from hen's egg yolk is an alternative method to cure the disease and also used to neutralize the pathogenic organism in humans. Antibody production in eggs is particularly advantageous because hens can be effectively immunized, antibodies are readily deposited in the yolk, and eggs are a convenient and inexpensive food source. ELISA kits were developed by using this IgY.

II.OBJECTIVES

- Purification and characterization of anti-*Salmonella enteritidis* antibodies.
- To evaluate this purity and specificity of the generated IgY using micro agglutination method.
- To detect the level of specificity by indirect ELISA method by the formulation of detection kit.
- To evaluate the Cross reactivity for the generated IgY.
- To develop the detection kit for checking the specificity by ELISA method.

III.MATERIALS AND METHOD

3.1 Experimental Animal

Twenty-one weeks old White Leghorn chickens in good health condition were obtained from Chandran poultry, Palladam.

3.2 Sample collection

Bacterial strain used for the present study is *Salmonella enteritidis*. The sample was collected from Bio line laboratory, RS Puram, Coimbatore.

3.3 Characteristics of organism

To check for purity of the culture, microscopic and biochemical characters of the strains were carried out by the following tests.

3.3.1 Gram staining test

The prepared smear was air dried and heat fixed. Crystal violet was flooded over the smear for one minute and drained. After washing Gram's iodine was added and left for one minute. The smear was then washed in 95% (V/V) ethanol for 30 seconds. It was counter stained with safranin for two minutes. After the slide was air drying, the smear was examined under oil immersion objective in a light microscope.

3.3.2Cultural characteristics of organism

The *Salmonella enteritidis* was cultured on Salmonella Shigella Agar, Bismuth Sulfite Agar, Hektoen enteric agar, XLD Agar and Nutrient agar plates. Then the *Salmonella enteritidis* culture was inoculated and Plates were incubated at 37ºC overnight and cultural characteristics of the colonies were studied after incubation.

3.3.3Biochemical test for *Salmonella enteritidis*

The biochemical test's for the confirmation of *Salmonella enteritidis* were carried out under sterile condition such as Indole, Methyl red, Voges proskauer, Citrate, Catalase test, TSI, Urease and Carbohydrate fermentation test.After incubation the reagents were added and result was observed.For catalase test the test organism was mixed with hydrogen peroxide.In Carbohydrate fermentation test Glucose, Lactose, Sucrose, Mannitol sugar was taken and test was carried.

3.3.4 Antibiotic suseptibility test

Muller hilten agar is most commonly used agar for the antimicrobial activity test. After that agar plate was prepared then it was innocolated with *Salmonella enteritidis* by making lawn culture using sterile swab.Then the Antibiotic discs(Co-trimazole,Chloromphenical,Amphicilin,Azythromycin) were placed on the agar and incubated at37 ^{0}c for 24 hours.

3.4 Preparation of whole cell antigen

Pure isolated colonies *Salmonella enteritidis* were grownup 5ml of Selenite F broth at 37ºC for overnight. Then the culture broth was centrifuged at 7000 rpm for fifteen mins. Take away the supernatant collect the pellet. Repeat this process at least three times and wash it with PBS until get a clear pellet. Formalin was added to it and kept overnight at room temperature. Again, the pellets were washed with PBS and stored under refrigeration.

3.4.1 Purity & Sterility testing of antigen

For the sterility checking, complete killing of the bacteria was tested by resuspending an aliquot of the cell pellet in PBS saline and plating of this suspension into nutrient agar and XLD agar. In purity checking, then the absence of bacterial growth in agar plates the immunization of chicken was carried out in aseptic condition.

3.5 Immunization of chickens

For 1st immunization, the five-month old white leghorn chickens were intra muscularly injected at the site of the breast muscles with prepared bacterial antigens. Booster doses were given. Eggs were collected at the intervals of three weeks from the initiation of immunization and checked for the presence of antibodies. Further, eggs laid by the chicken under the test were collected regularly and stored at 4ºC.

3.6 Purification and concentration of anti-*Salmonella enteritidis* antibodies from Egg Yolk

3.6.1 Separation of Egg-Yolk

Under strict aseptic techniques the egg yolk was separated from white and was washed with water to remove as much albumin possible. The yolk membrane and any remaining egg white will stick to the tissue paper. The yolk sac was discarded. The amount of yolk obtained was measured. Then a continuous flow of 25mM phosphate buffer was maintained until all the un-retained protein came out. IgY was eluted with 250mM phosphate buffer pH 8.0.

3.6.2 Purification of IgY

The egg yolk antibodies were purified by the method of Polson *et al.*, (1980). To the 20ml of egg yolk, an equal amount of buffer "S" (10mM phosphate, 100mM NaCl, pH 7.4 containing 0.01% sodium azide) was added to the yolk and stirred. To this mixture 10.5% PEG 8000 in buffer "S" was added to a final concentration of 3.5%. The mixture was stirred for 30 minutes at room temperature. The stirred mixture was centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered through Whatman filter paper. The PEG was added to the supernatant for final concentration of 8.5%. The mixture was stirred and centrifuged at 10,000 rpm for 20 minutes. The pellet was collected and the 10ml buffer-"S" was added to make final concentration of 12.5% PEG. The mixture was stirred thoroughly for 30 minutes at room temperature and centrifuged at 10,000 rpm for 20 minutes. The pellet was taken and 800 µl of PBS was added and mixed it well.

3.6.3 Purification of IgY fraction by dialysis

The egg yolk antibodies were collected and mixed well immediately the antibodies were packed in dialysis bags for further purification process. The activation of cellulose membrane was facilitated by the membrane was cut into pieces of required and convenient length and allowed to boil for 10 minutes in a large volume of 2% (w/v) sodium bicarbonate and 1mM of EDTA (pH 8.0). The tubing was rinsed thoroughly in distilled water. Again, it was allowed to boil in 1mM EDTA (pH 8.0). The tubing was cooled down and stored at 4°C. Care was taken that the tubing was thoroughly submerged. After this step, the tubing was handled with gloves. Before use, the tubing was washed inside and outside with distilled water. The pooled IgY fraction obtained from egg yolk was transferred to an active dialysis bag. The contents were pooled into the dialysis bag and clipped with dialysis bag clips after including some air and twisting the open end of the dialysis bag. The bags containing pooled contents were dispensed into the respective NaCl solution and kept overnight for dialysis process. After the bags were transferred into phosphate buffer solution.

3.7 Packing of chromatography column

For purification of immunoglobulin, the column size 2x30cm was used. The burette was first cleaned well and it was packed first with glass wool to form an even bed and a rubber tube with pinch-cock was attached to the tip of the burette. The column was fixed to stand in vertical position. The silica gel was poured into the column along the sides to avoid air bubbles and was allowed to settle. Once the column was set, it was equilibrated with 25mM phosphate buffer (till the out flow of buffer showed pH 8.0).

3.8 Sample application and elution

Once the column was equilibrated (25mM phosphate buffer, pH 8.0) the level of buffer in column was allowed to run down to the matrix and the outlet was closed. The immunoglobulin IgY sample layered on the top of the column and was allowed to run till all the sample had entered the bed.

3.9. Collection of blood

The blood collection was performed after the second dosage of antigen was given. By using sterile syringe, 2ml of blood was collected from the hen for the comparison of IgY antibody concentration in Egg yolk and serum

3.10. Separation of serum

The collected blood was undergone centrifugation. The serum was present in the top of the layer and other cells were settled in a tube. Then the serum alone transferred into new Eppendorf tube by using micropipette.

3.11. Rapid agglutination test

The specificity of anti-Salmonella antibodies of the chicken egg yolk was determined by Rapid Slide Agglutination Test (RSA). Test was done on the plastic strip; 20µl of antigen and 20µl of IgY were placed on a strip and mixed thoroughly by stirring with the help of applicator stick. Then the slide was observed for the appearance of agglutination within 2 minutes.

3.12. Estimation of protein content in IgY fraction by Lowry et al., (1951)

The total protein content was estimated by the method described by Lowry et al., (1951). [14] A quantity of 10mg Bovine Serum Albumin (BSA) was dissolved in 10ml of distilled water and used as Protein stock solution. To a series of clean test tubes 0.2 – 1.0 ml of BSA (Protein stock solution) was added and made up to final concentration of 5 ml with distilled water. From these dilutions 0.2ml was taken in to different test tubes and 2ml of Alkaline Copper Sulphate solution was added to every test tubes and incubated for 10 minutes at room temperature. After incubation, 2ml of Folin-Ciocalteau reagent was added followed by 1 ml of test sample was poured in same each test tubes incubated under dark condition for 30 minutes at room temperature. Finally, the optical density (OD) was measured at 660nm. The OD values of IgY were compared with standard graph. After this total protein content was estimated by UV-Vis spectrophotometer at 260nm.

3.13. Protein Profile of IgY

Protein profile of IgY antibodies were analyzed by Sodium Dodecyl Sulphate Ploy Acrylamide Gel Electrophoresis (SDS-PAGE) as described by Laemmli (1970). According to Laemmli (1970) the proteins are resolved with 10% (W/V) polyacrylamide separating gel and 4% (W/V) poly acryl amide stacking gel at 250V and 10mA.Equal ratio of prepared bacterial antigens (30µl) and sample treating buffer (30µl) were mixed well and loaded into sample wells. A wide range molecular weight (6.5-205 KDa) marker was also run along with the proteins. The characteristic protein pattern for the IgY antibodies can be visualized after sliver staining. The same protocol followed for the Serum.

3.14. Concentration of antibodies

The eluted IgY fractions were pooled together and concentrated. The dilutions were made from 1:10 to 1:1, 00,000. In 1:10 dilution 200 µl of Crude IgY was mixed with 800µl of PBS buffer. From this mixture 200µl was taken and transferred into next dilution tube which contains 800µl of PBS. This is known as 1:100 dilution repeat this process up to 1, 00,000 dilution from that mixture 200µl was taken and discarded.

3.15. Titration of antibody by ELISA

The antibody titer potency of each serums and IgY fractions obtained above was determined by the following modified ELISA. The particulate *Salmonella enteritidis* antigen was dispensed at a concentration of 5µg/100µl in phosphate buffer. The resultant dispersion was coated into the individual wells of a 96-well plate (Polyvinyl ELISA plates), at a rate of 100µl per well and incubated at 4°c overnight. The solution was then discarded and unbound antigens in the wells were removed by washing with PBS-T for 3 times. The nonspecific binding sites were blocked by adding 200µl per well of1% bovine serum albumin in PBS and incubating the plates at 37°C for 1 hour. Plates were subsequently washed with PBST and the individual wells of each plate were then added with 100µl aliquots of Egg yolk antibodies (IgY) sat appropriate dilutions, followed by reaction at37°C for 1 hour. In control wells PBS were served as respective control. After the reaction the plates were washed three times with PBS-T. As a secondary antibody biotinated rabbit anti chicken IgY coupled to horseradish peroxidase (Genie Pvt. Ltd, Bangalore) was added at the rate of 100µl per well and the plates were incubated for 1 hour at 37°C. The plates were then washed 3 times with PBST. The antibody titer was determined by adding 100µlof freshly prepared substrate solution to wells of every plate and followed by reaction at room temperature in dark for fifteen min. The reaction was terminated by adding 50µl of terminating solution (4N H2SO4). The absorbance of the well was measured in an ELISA reader at OD490.The same protocol followed for the Serum.

3.16. Micro Agglutination Method

In this micro agglutination method 50µl of antigen was taken in each tube from 1:20–1:640 concentration. Before taking the antigen, it was mixed with 20µl of methylene blue. Then the 50 µl IgY antibodies were taken added into first tube and from that first tube 50µl was taken and added to second tube. Continue this process until last tube. From that tube 50µl was taken and discarded. Then the OD value was taken at 470nm under Spectrophotometer.

3.17. Growth inhibition assay for *Salmonella enteritidis*

The fresh *Salmonella enteritidis* culture broth was prepared and kept for incubation at 37^0 c for 24 hours. Then the 24 hours culture was taken and 1 ml is poured into every 11 tubes. First tube is control tube without antibody. In second tube 0.1µl of IgY was added, in third tube 0.2µl of IgY was added. Continue this up to last tube. In last tube 1.0µl of IgY was poured. After the addition of antibodies, the tubes were kept in incubation at 37^0 c for 24 hours. The OD value was taken at 470nm

3.18. Cross reactivity

Cross reactivity test was performed to check the specificity of IgY. This test was done by using the u bottom plate. 50 µl of IgY antibody was poured into each fives wells of u bottom plate. 50µl of *Salmonella enteritidis* antigen was added in the first well while the other wells were poured with other antigens (*Shigella* Ag, *Streptococcus* Ag, Candida *albicans* Ag and Gliadin Ag). After that the plates were observed and OD value was taken in ELISA reader at 650 nm and the results were tabulated.

3.19. Development of ELISA kit

3.19.1 Ag coated ELISA kit (Direct ELISA)

Readymade ELISA kits were prepared. In direct ELISA plate *Salmonella enteritidis* Ag was coated and kept for overnight. If Ag was coated means patient's serum were tested in that plate. In ELISA titer plate 10µl of *Salmonella* antigen was coated and left overnight at 4^0C.The following day, after washing with PBS buffer, wells were blocked with blocking solution for one hour at room temperature and washed again with PBS. Then this plate was kept in storage at 4^0C.

3.19.2 IgY Coated ELISA kit (Indirect ELISA)

In indirect ELISA plate IgY was coated and kept for overnight. If Ab was coated means patient's stool samples were tested in that plate. In ELISA titer plate 10µl of anti-Salmonella enteritidis was coated and left overnight at 4^0C.The following day, after washing with PBS buffer, wells were blocked with blocking solution for 1 hour at room temperature and washed with PBS. Then this plate was kept in storage at 4^0C.

IV.RESULTS AND DISCUSSION

4.1 Characterization of *Salmonella enteritidis*

4.1.1 Gram staining

The gram stained smear of the strain *Salmonella enteritidis* showed gram negative, rod shape measuring of 2-5µm long by 0.5-1.5µm wide. Motile bacteria that don't form spores.

4.1.2 Cultural characteristics

Salmonella enteritidis

The isolated bacterial cultures were grown on Nutrient agar. The colony morphology was studied on Nutrient agar, Salmonella Shigella agar, Bismuth Sulfite Agar and XLD. S*almonella enteritidis* on nutrient agar showing smooth colonies. Salmonella Shigella agar is a selective medium for the isolation of gram-negative bacteria *Salmonella enteritidis*. On SS agar *Salmonella enteritidis* produce smooth and opaque colorless colonies. In XLD agar plate this organism gives Black color colonies. On BSA plate *Salmonella enteritidis* produce black color colony with metallic sheen.

Table 1: Characterization of colonies

S.NO	NAME OF THE MEDIUM	CHARACTERIZATION OF COLONY
1	Nutrient Agar	Smooth colonies
2	Bismuth Sulfite Agar	Black color with metallic sheen
3	Salmonella Shigella Agar	Smooth and opaque colorless colonies

4.1.3 Biochemical characteristics

Biochemical characteristics of the bacterial strain were determined by using various biochemical tests like catalase, carbohydrate test, MR-VP test, Indole test, Urease test, TSI test etc., their results were recorded in the following table.

Table 2: Biochemical test result table

S. No	BIOCHEMICAL TEST	RESULT
1	Indole test	Negative
2	Methyl Red test	Positive
3	Voges-Proskauer test	Negative
4	Citrate test	Positive
5	Catalase test	Positive
6	Urease test	Negative
7	Triple Sugar Iron test	Red slant, black butt H_2S produced
8	Carbohydrate test	
	1)Glucose	Positive
	2)Lactose	Negative
	3)Sucrose	Negative
	4)Maltose	Positive

4.1.4 Antibiotic susceptibility test

Table 3: Antibiotic sensitivity result

Antibiotic disc	Zone of inhibition(Radius)	Susceptibility
Co-Trimaxazole	1.6cm	Sensitive
Chloromphenicol	0.75cm	Intermediate
Amphicillin	1.4cm	Sensitive
Azythromycin	1.8cm	Sensitive

4.2. Generation of Antibodies in Hen

The 21-week old white leghorn Hens were immunized intramuscularly with prepared bacterial antigens to generate anti-*Salmonella enteritidis* with two-week intervals. The eggs were collected and antibodies were separated from the egg yolk. Eggs were collected from fourth week of immunization and stored at 4°C.

4.3. Isolation and Purification of Antibodies

The purification method of chicken egg yolk antibodies facilitated by PEG. The pellets were desalted by dialysis for purification. The egg yolk antibodies were further purified by column chromatography and immunoglobulin fractions were recovered. The recovered antibodies were detected by Protein estimation.

4.4. Protein estimation

The total protein concentration of purified IgY was estimated by using UV visible spectrophotometer. Total protein concentration of serum antibody was found to be 0.45 ml and protein concentration of egg yolk IgY was found to be 0.41ml. Comparatively serum and egg yolk antibody will be higher than the control egg.it determine it produce high amount of antibodies in immunized egg against *Salmonella enteritidis* antigen**.**

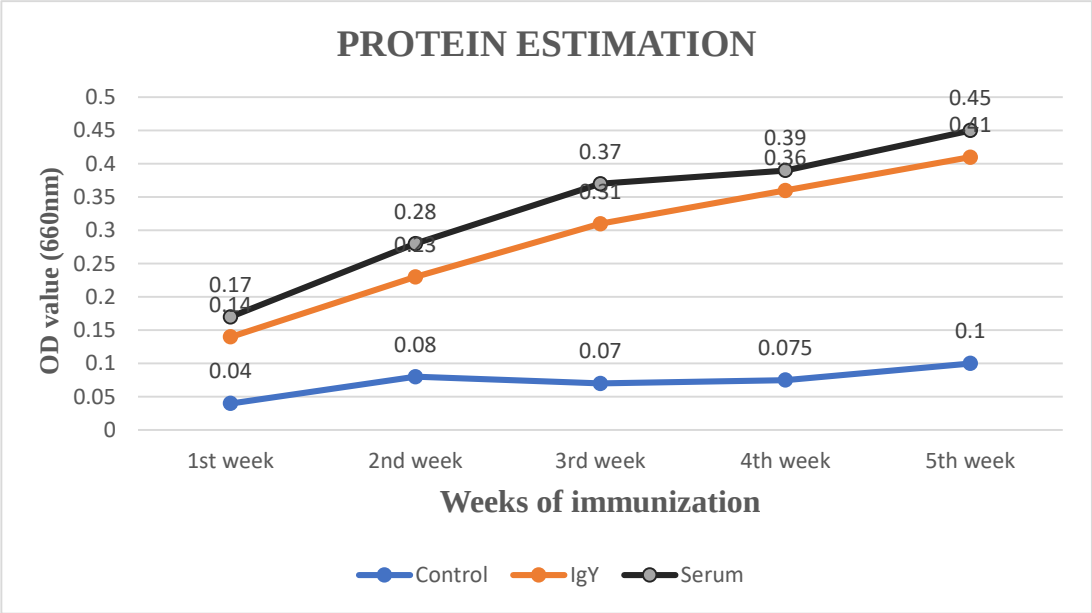


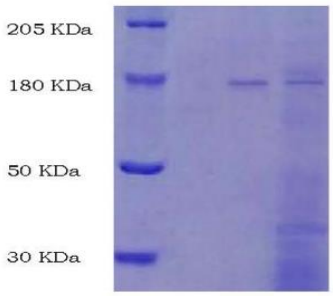
Figure 1:Estimation of Anti-*Salmonella enteritidis*

4.5. Rapid slide agglutination test

Specificity of anti-*Salmonella enteritidis* antibodies in the egg yolk from immunized laying chickens was determined by Rapid slide agglutination Test (RSA). Appearance of agglutination within 2minutes, when the antigen was mixed with the corresponding IgY on plastic strip, revealed that the antibody generated in the immunized chicken which was purified as IgY-extracts from eggs which were specific against to their respective antigens.

4.6. IgY profiling by SDS PAGE

In this SDS Page it forms ladder with ranges. Each band in the ladder is known molecular weight. The samples can be determined from these well-known weights. The chicken egg yolk antibodies and its molecular weight was determined by Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) using 10% polyacrylamide gel at 100V and 10 mA according to the method of Laemmli (1970). The purity of IgY increased to 95% after several purification steps. The electrophoretic pattern of gel filtration fraction with the standard IgY, which was 90% pure . IgY is composed of heavy chains about 205KDa and light chains about 30KDa.



- **Lane 1-Marker**
- **Lane 2- IgY fraction by 14th day of *S. enteritidis***
- **Lane 3- IgY fraction by 28th day of *S.enteritidis.***

Figure 2: SDS PAGE of IgY

4.7. Estimation of antibody titer by ELISA

The antibody titer of each IgY fractions obtained above was determined Indirect ELISA as described by Lee *et al.*, (2002). The antibody titer increases at the time of booster injections, even a minute increase in antibody titer can be traced by this assay. The comparative results show that the antibody titer potencies changes in the courses of immunization. Antibody titer was very low at 0th day egg, then there was a steady increase in titer at consecutive booster immunization and reached its peak at 49th day (Plate 2b & Figure 4). After 49th day there was a steady plateau in antibody titer till 70 th day.

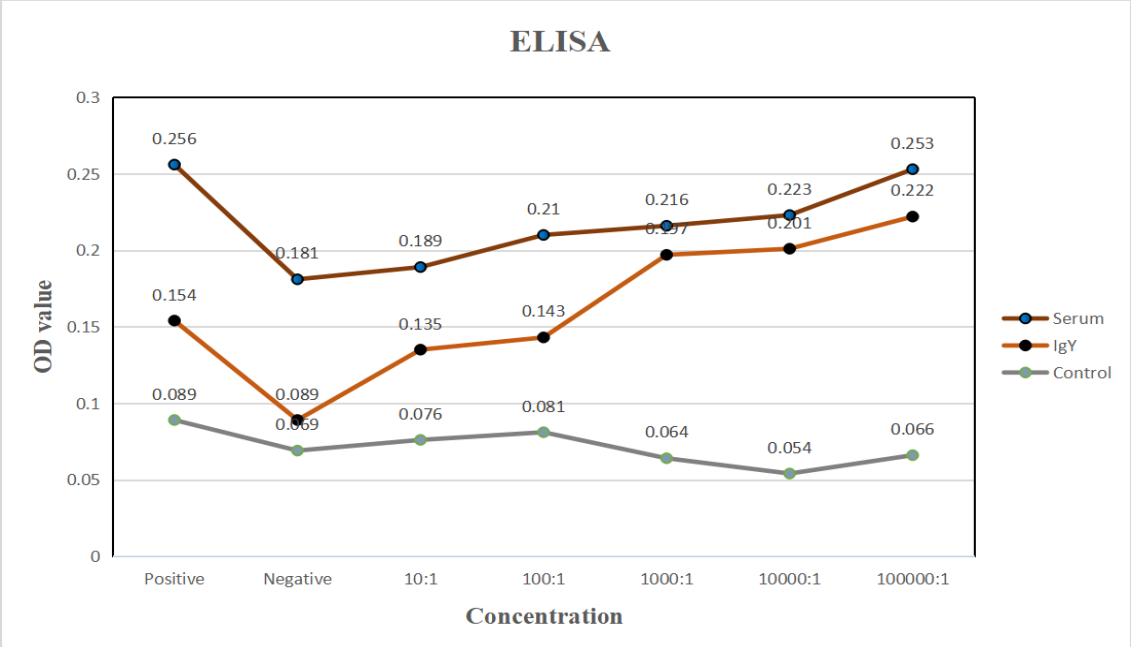


Figure 3: IgY against *S.enteritidis* ELISA result

The level of Antibodies specificity was estimated by indirect ELISA. In 1:1,00,000 concentration it gives 0.222 at OD450.

4.8. Growth inhibition assay for *Salmonella enteritidis*

The Minimum inhibition concentration depends upon the level or concentration of antibody which we added to test sample. The smallest concentration of an antibody that inhibits the growth of *Salmonella enteritidis* is 86%. This value is known as Minimum inhibitory rate.

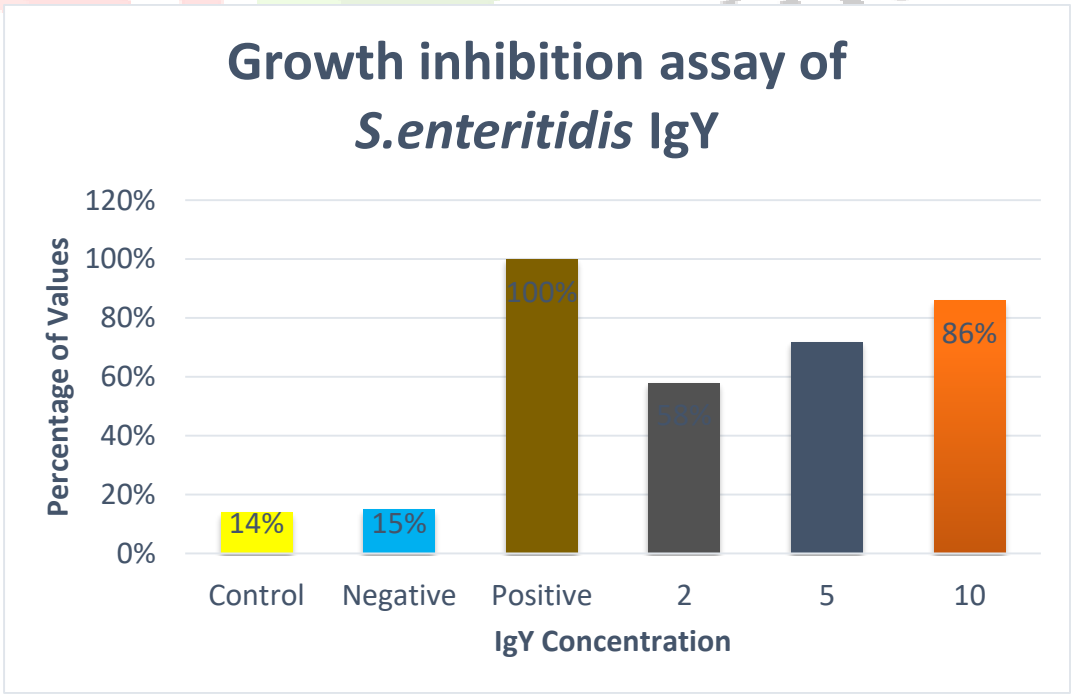


Figure 4: Growth inhibition assay

4.9. Micro agglutination

Micro agglutination test was performed in a microtiter plate. There are six different concentration was taken in those concentrations 1:20 gives a high rate of agglutination. when the dilution rate was increased agglutination rate was decreased. In 1:640 the level of agglutination is low.

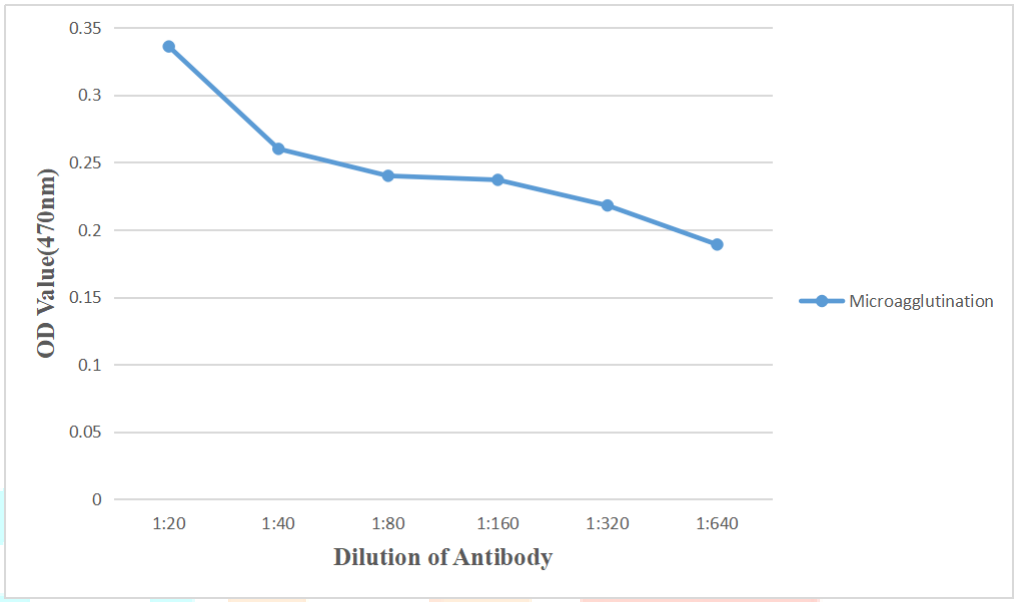


Figure 5: Microagglutination OD value graph

4.10. Cross Reactivity test

In Cross reactivity it gives agglutination that is very specific to *Salmonella enteritidis* antigen. The IgY was tested with other type of antigens but other antigens give minimal amount of reactivity because it is polyclonal antibody so it gives minimal specificity to other antigens and more specific to *Salmonella enteritidis* antigen. It gives 3.051 OD at 650nm.

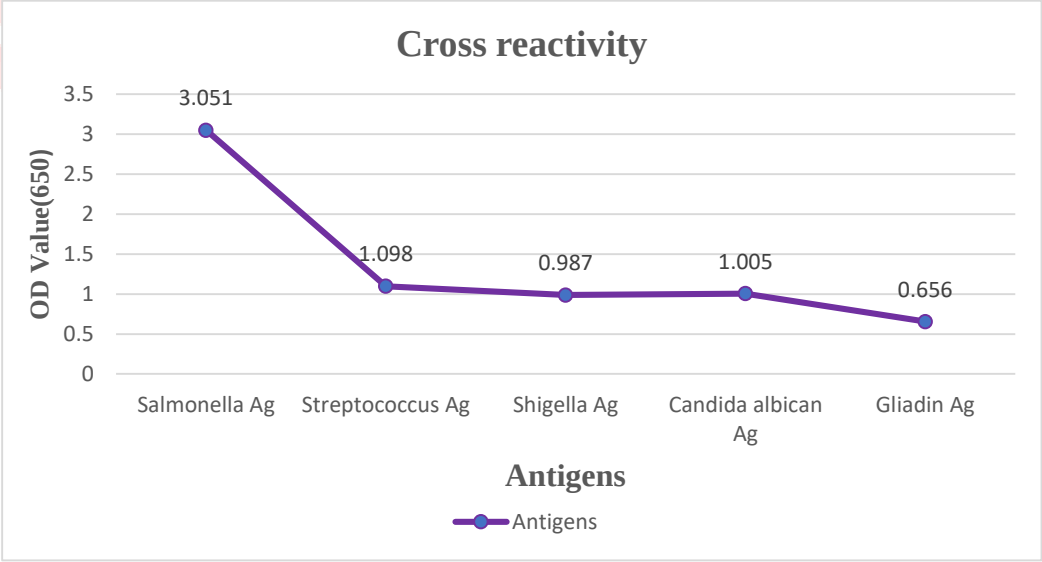


Figure 6: Cross reactivity result

4.11. Development of ELISA kit

The present study developed ELISA kit is capable of detecting specific antibodies against *Salmonella enteritidis* antigens and specific antigens against the antibodies causing gastrointestinal infection. This readymade ELISA kit can be used for the serological applications and diagnosis of gastrointestinal infections. This kit should store under the 4^0C. It can be stored for almost 6-10 months.

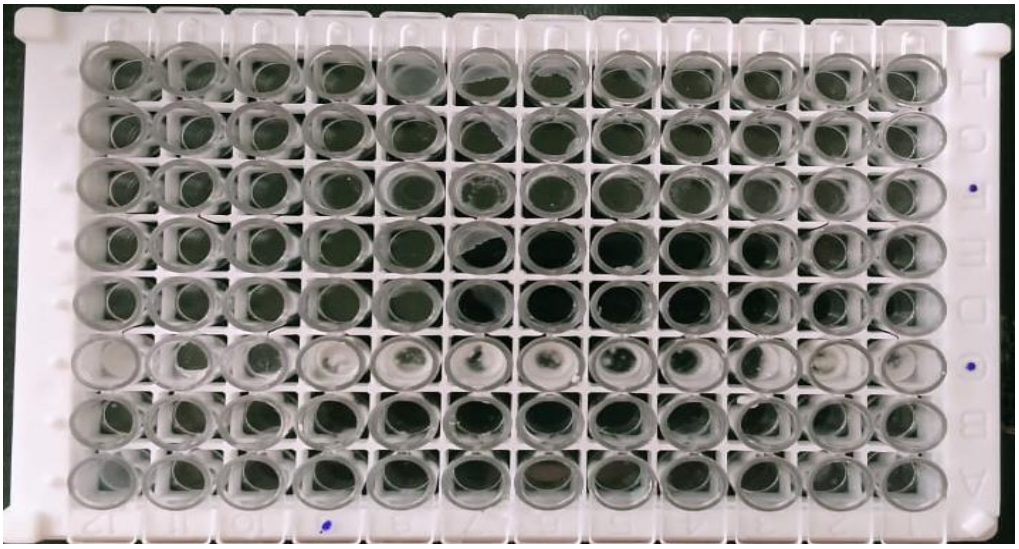


Figure 7*: Salmonella enteritidis* antigen labeled detection kit

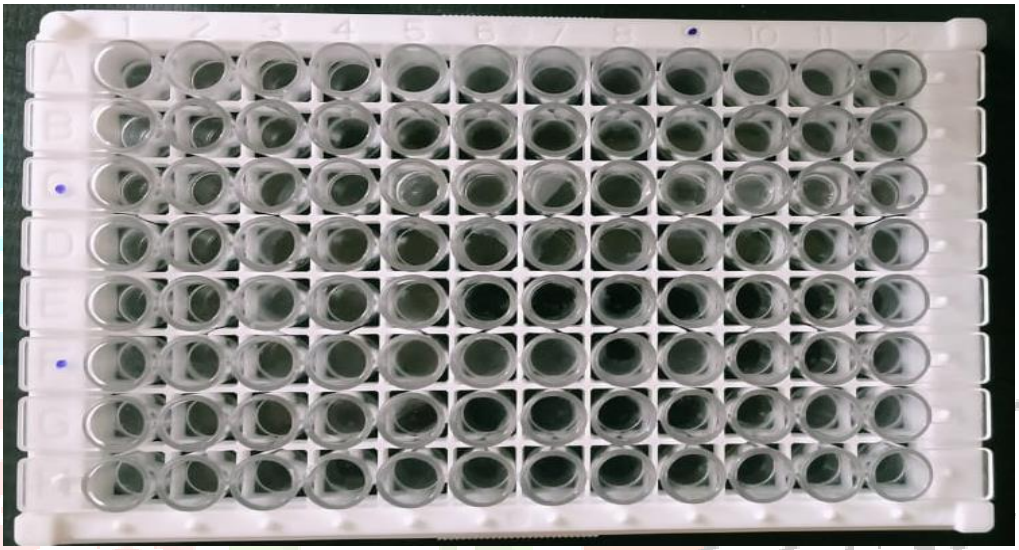


Figure 8*: Salmonella enteritidis* IgY labeled detection kit

V.CONCLUSION

The present study was undertaken to generate antibodies agsinst *Salmonella enteritidis* which cause Gastrointestinal infecction.The chicken were immunized with prepared *Salmonella enteritidis* antigen.Subsequent booster doses were given periodically at one week interval . The eggs were collected and stored at 4^0C.

Purification and characterization of the IgY was done by collecting the egg from the immunized chicken.The eggs were centrifuged and dialysed for the purification process. Purification of IgY antibodies from collected eggs was done according to the method of Polson *et al.*, 1980. In protein estimation the concentration of IgY was increased based on the week of eggs. In 5^{th} week it gives 0.41mg/ml concentration .That is the high rate of IgY in collected elusion.

Purity and specificity of the generated IgY is the most important methods to carried. For this purity and specificity microagglutinaion test was performed . In this test 1:20 gives the high rate of agglutination.That is 0.336 at OD^{470}nm. It states that this IgY is highly specific to that *Salmonella enteritidis* antigen.It also determined the purity of the antibodies.The specificity of this IgY was detected by indirect ELISA method.

Cross reactivity is the most important process to check the specificity and the evaluation of generated IgY.In this test the Anti-Salmonella enteritidis was allowed to react with Antigens.This IgY react with specific antigen only.This is high rate of reaction so it concludes that this IgY is highly specific to that Antigen.The direct and indirect ELISA kits were prepared and developed. In direct ELISA the *Salmonella* antigen was coated in titer plate so the patients serum will test in this plate.In indirect ELISA IgY antibody was coated here patients stool sample will collect and test in this plate.

VI.ACKNOWLEDGMENT

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REFRENCES

1. Chansarkar N.L., 1998. Studies on structural stability of hen's egg yolk immunoglobulin (IgY). PhD Thesis: University of British Columbia (Canada).
2. Carlander D., Kollberg H., Wejaker P. and Larsson A. 2002. Peroral immunotheraphy with yolk antibodies for the prevention and treatment of enteric infections. *Immunol. Res*. 21: 1–6.
3. Lee E.N., Sunwoo H.H., Menninen K. & Sim J.S., 2002.*In vitro* studies of chicken egg yolk antibody (IgY) against *Salmonella Enteritidis*, *Salmonella Typhimurium*. *Poult. Sci*., 81, 632-641.
4. Polson A., Von Wechmar M.B. and van Regenmortel M.H.V. 1980. Isolation of viral IgY antibodies from egg yolks of immunized hens. *Immunol.Commun*. 9: 475.
5. Polson A *et al*., 1990. Isolation of IgY from the yolks of eggs by a chloroform polyethylene glycol procedure. *Immunol. Invest*., 19(3), 253-258.
6. Polson A. *et al.*, 1985. Improvements in the isolation of IgY from the yolks of eggs laid by immunized hens. *Immunol. Invest*., 14(4), 323-327.
7. Gordon G, Moran G, Ayala L,Seqqat R, Fernandez R and Torres M. 2016.Generatioon and characterization of IgY NTIBODIES FROM Lhomann Brown Hens Immunized with Salmonella spp, For their Subsequent Applicaion in Nanotherapy.8 :3.
8. Bruno De Meulenaer & Andre Huyghebaert,2001. Isolation and purification of Chicken Egg yolk immunoglobulin :A review , Food and Agricultural immunology,13 :4,275-288.
9. Lang Zha, Shari Garrett, and Jun Sun, 2019.Salmonella infection in Chronic Inflammation and Gastro-intestinal Cancer.7(1) :28.
10. Jonathan R Kurtz, J. Alan Goggins, and James B. McLachlan 2017.Salmonella infection : interplay between the bacteria and host immune system. 190 :42-50.
11. Shu-Kee Eng, Priya Pusparajah,Nurul-syakima Ab Mutalib, Hooi-Leng ser, Kok-Gan Chan & Learn-Han Lee.2015 Salmonella : A review on pathogenesis, epidemiology and antibiotic resistance.284-293.
12. Anjila Maharrajan, Puspa Raj Khanal, and narayan Prasad Parajuli. 2017 Enteric fever caused by *Salmonella entrica* serovars with reduced susceptibility of Fluoroquinolones at a Community Based Teaching Hospital of Nepal.
13. John A. crump, Maria Sjolund-Karlsson,Melita A. Gordon,Christopher M. Parry 2015.Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive *Salmonella* infections.
14. Nanacy F. Crum Ciaflone, MD MPH.Salmonellosis and the GI Tract: More than Just Peanut Butter. (2014);424-431.
15. Min-Soo Kim, Yeo-Sang Yoon,and Do-Young Yum .A Study on the Prevention of Salmonella Infection by Using the Aggregation Characteristics of Lactic Acid Bacteria .(2015);31(2):219.
16. Hung-Ming Chen , Cheng-Hsun Chiu.Nontyphoida Salmonella Infection: Morphology, Clinical Features, and Antimicrobial Therapy.(2013);147-152.
17. Wala Ahmad Amro, Wael Al-Qaisi,Fawzi Ai-Razem.Production and purification of IgY antibodies from chciken egg yolk.(2018);99-103.
18. R K Gast et al., Avian Dis.The relationship between the magnitude of the specific antibody response to experimental Salmonella enteritidis infecction in laying hens and their production of contaminated eggs.(2001);45(2):425-31.
19. R K Gast et al., Avian Dis. Production of Salmonella entritidis- contaminated eggs by experimentally infected hens.(1990);438-46.
20. Christopher R Braden. Salmonella entrica serotype Enteritidis and eggs: a national epidemic in the United states.(2006);43(4):512-7.
21. Saleh mohammed Jajere.A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance.(2019);12(4):504-521.
22. Zoe R.Howard ,Steven C.Rickle.*Salmonella enteritidis* in shell eggs:Current issues and prospects for control.(2012);755-764.
23. R Chalghoumi et al.,Production of hen egg yolk immunoglobulins simultaneously directed against *Salmonella enteritidis* and *Salmonella typhimurium* in the same egg yolk.(2008).
24. E.P.V. Pereira, M.F. van Tilburg and M.I.F. Guedes.Egg yolk antibodies (IgY) and their applications in human and veterinary health: A review .(2019);73:293-303.
25. Chrtsoula-Evangelia Karachaliou, Evangelia Livaniou. Development of a specific IgY-based ELISA for prothymosin alpha, a bioactive polypeptide with diagnostic and therapeutic potential.(2019).
26. Shimizu M, Nagashima H, Sano K, Hashimoto K, Ozeki M, Tsuda K et al. Molecular stability of chicken and rabbit immunoglobulin G. Biosci Biotechnol Biochem 1992; 56(2):270-274.
27. Rose ME, Orlans E, Buttres N. Immunoglobulin classes in the hen's egg: their segregation in yolk and white. Eur J Immunology 1974; 4:521-523.
28. Tressler RL, Roth TF. IgG receptors on the embryonic chick yolk sac. J Biol Chem 1987; 262(32):15406-15412.
29. Woolley JA, Landon J. Comparison of antibody production to human interleukin-6 (IL-6) by sheep and chickens. J Immunol Methods 1995; 178(2):253-265.
30. Brambell R. The transmission of passive immunity from mother to young. Frontiers in biology 1970; 18:20-41.
31. Gassmann M, Thommes P, Weiser T, Hubscher U. Efficient production of chicken egg yolk antibodies against a conserved mammalian protein. FASEB J 1990; 4(8):2528-2532.
32. Hassl A, Aspock H. Purification of egg yolk immunoglobulins. A two-step procedure using hydrophobic interaction chromatography and gel filtration. J Immunol Methods 1988; 110(2):225-228.
33. Losch U, Schranner I, Wanke R, Jurgens L. The chicken egg, an antibody source. Zentralbl Veterinarmed [B] 1986; 33(8):609-619.
34. Nishinaka S, Suzuki T, Matsuda H, Murata M. A new cell line for the production of chicken monoclonal antibody by hybridoma technology. J Immunol Methods 1991; 139(2):217-222.
35. Svendsen BL, Hau J. Chicken eggs in polyclonal antibody production. Scand J Lab Anim Sci 19960; 23(1):85-91.