

Detection of Egg Yolk Immunoglobulin Y; a Potential Source of Anti-*Escherichia coli*

Enayatollah Kalantar^{1,2}; Mohammad Mehdi Soltan Dallal²; Laya Kafami Khorasani³; Koursh Kabir⁴; Monireh Zenolabedini Zamani^{2,*}

¹Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, IR Iran

²Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, IR Iran

³Department of Microbiology and Immunology, Alborz University of Medical Sciences, Karaj, IR Iran

⁴Department of Social Medicine, School of Medicine, Alborz University of Medical Sciences, Karaj, IR Iran

*Corresponding author: Monireh Zenolabedini Zamani, Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +982188992971, Fax: +982188992971, E-mail: manasept63@gmail.com

Received: January 24, 2015; Revised: February 14, 2015; Accepted: February 23, 2015

Background: *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases. Increasing occurrence of antibiotic resistance would complicate the treatment of such diseases.

Objectives: The aim of this study was to detect the egg yolk immunoglobulin Y as a potential source of anti-*Escherichia coli*.

Materials and Methods: This study was conducted by using killed *E. coli* with formaldehyde as antigen. Chickens were immunized with 500 µL of antigen diluted in equal volume of Freund's complete adjuvant (FCA). Booster injections of increasing concentrations of antigen were given to raise the antibody level in serum. The specificity of antibody was confirmed by ELISA method.

Results: Chicken egg yolk antibodies (IgY) were raised against *E. coli* in the serum after injections. Up to 1:10000 dilution antibodies were determined in serum, which shows high amount of antibodies are produced in immunized chickens.

Conclusions: Further studies have to be conducted to evaluate the potency of these antibodies in treatment of variety of diseases. The outcome of this research work would be an alternative to the current antibiotic treatments.

Keywords: *Escherichia coli*; Serum; Enzyme-Linked Immunosorbent Assay

1. Background

Escherichia coli is a normal resident of the intestines of humans and most animals. Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases, such as diarrhea (1-3). Furthermore, *E. coli* is one of the major ubiquitous pathogen which causes the food poisoning in notable manner (4).

There have been tremendous efforts to produce numerous new antimicrobial agents to control the bacterial infections; because antibiotic therapy fails in most of bacterial infections because of the increase in antibiotic resistance among bacteria (5, 6); therefore, increasing incidence of antibiotic resistance would additionally make problems the treatment of such diseases thus, it is important to look for new therapies to prevent such disease.

2. Objectives

Recently, scientists focused and intentioned in use of IgY antibodies from eggs of chickens against pathogens for immunotherapy and diagnosis because it has a unique biological activities (7). For prevention of bacterial infections, using IgY has several advantages like it can be easily

isolated from egg yolk by the water dilution method on a large scale without using any chemicals or organic solvents. Furthermore, the antibody yield of an egg-laying hen is more than others and more importantly IgY from mammalian blood is time-consuming and expensive (8). Based on the above reports, the present study was formulated with the following objectives;

- i. To produce immunoglobulin Y (IgY) against *E. coli*.
- ii. To identify anti-*Escherichia coli* immunoglobulin Y in serum by ELISA method.

3. Materials and Methods

3.1. Experimental Animals

Four 50 week old, white highline chickens were obtained from aviculture and kept in animal house, School of Medicine, Alborz University of Medical Sciences, Karaj. They were used in the study for the production of anti-*Escherichia coli* antibodies (IgY).

3.2. Identification of *Escherichia Coli*

Escherichia coli were obtained from Microbiology Department, School of Medicine, Alborz University of Medi-

cal Sciences, Karaj. The *E. coli* were isolated from children with acute diarrhea (9).

3.3. Preparation of Antigen

Escherichia coli were grown in Brain Heart Infusion Broth overnight at 37°C. Bacteria was harvested by centrifugation (15 minutes, 3000 rpm), washed three times with PBS (pH 7.2), and re-suspended in PBS at a density of 108 cells/mL by comparing 0.5 McFarland and cells OD at 600 nm was recorded.

For killing the *E. coli*, formaldehyde was used at concentration of 10% (vol/vol), and suspension was hold at 4°C for 16 hours. In order to remove the formaldehyde, *E. coli* were washed twice with PBS and re-suspended in sterile PBS. By culturing the *E. coli* on MHA for 24 hours at 37°C, complete killing was confirmed and the suspension was stored at 20°C (10).

3.4. Development of Anti-*Escherichia coli* Antibodies in Chickens

Chickens received four injections, one primary injection and three boosters. For the main injection, 500 µL of antigen was emulsified with an equal volume of Freund's complete adjuvant (FCA) using Herbert et al. procedure (11). Then the solution was injected intramuscularly at one site of breast muscle of chickens. Three booster injections of antigen with Freund's incomplete adjuvant (FIA) were given at 14 days interval by the same route of administration. Two mL blood sample was collected from chickens before immunization as preimmune sera.

3.5. Serum Separation

Serum was separated by centrifugation for 10 minutes at 3000 rpm and stored at 20°C until use.

3.6. Serum's Antibody Titer by Indirect ELISA

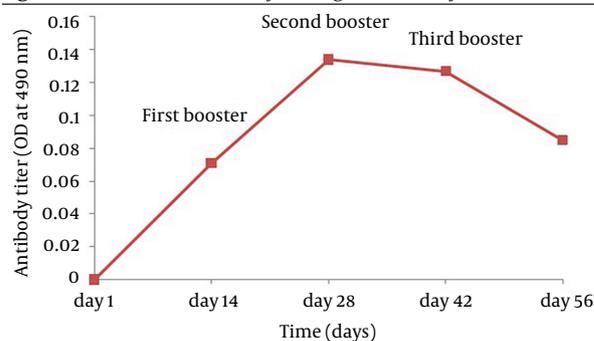
The antibodies titer was determined by an enzyme-linked immunosorbent assay (ELISA) procedure as described by Sunwoo et al. (12).

4. Results

The activities of anti-*E. coli* antibody are detected by ELISA in serum from laying hens as shown in Figures 1, 2 and 3.

The titer of the specific antibody produced in immunized hens increased rapidly from the first injection and reached a peak after second booster injection at the fourth week and was maintained stable for two weeks. However it decreased after last booster injection that this subject shows in order to achieve the stability in the titer of antibody, require repeated booster injections (Figure 1). Furthermore comparing serum dilutions with control group (preimmune sera) indicate that up to 1:10000 dilution antibodies present in serum, which shows high amount of antibodies are produced in immunized chickens (Figures 2 and 3).

Figure 1. The Produced Antibody Titer Against *E. coli* by ELISA.



The titer of the specific antibody increased after first injection.

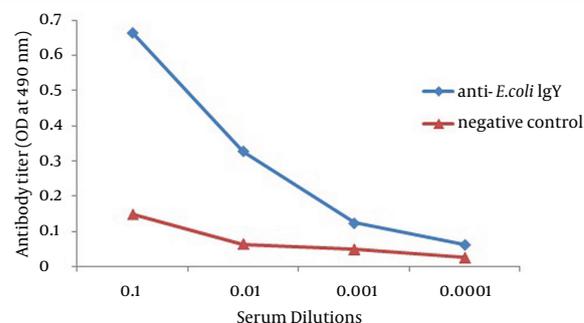
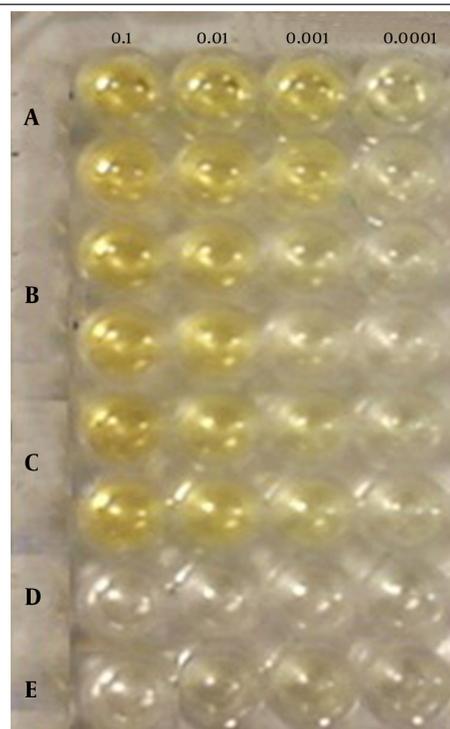


Figure 2. Comparing Serum Dilutions With Control Group (Preimmune Sera), up to 1:10000 Dilution Antibodies Present in Serum

Figure 3. Titration of Antibody by ELISA in Dilutions of 1:10, 1:100, 1:1000, 1:10000



Preimmune sera was served as negative control. The positive reaction shows up to 1:10000 dilution. A: Serum Of Chicken 1, B: Serum Of Chicken 2, C: Serum Of Chicken 3, D: Blank, E: Negative Control.

5. Discussion

Administration of IgY to host pathogens is an attractive approach to establish protective immunity, especially against gastrointestinal pathogens in human; furthermore, scientists reported that, IgY has been used for suppression of growth of food-borne pathogens (13). In addition, the specificity of avian antibodies often differs significantly from that of comparable mammalian antibodies. An important consequence of this is that the Rheumatoid Factor (RF) cannot bind with IgY. RF is a major source of interference in many immunoassays, being an autoantibody that reacts with the Fc portion of mammalian IgG. The disease usually associated with RF is rheumatoid arthritis, but RF is also present in serum from patients with many other diseases. Most immunoassays use mammalian polyclonal or monoclonal antibodies, which are prone to RF binding, thus giving false positive results. However, RF is not able to bind to the IgY molecules; therefore, chicken antibodies can be useful in assays (e.g. nephelometry, latex-aggregation, or ELISA) where RF could interfere. Apart from the biological differences (RF recognition) the difference in molecular structure gives rise to a variety of physico-chemical differences (solubility, affinity for adsorption, isoelectric point, etc.). The heavy chains (H) of IgY are larger than, and antigenically distinct from, those of mammalian IgG (14).

In the present study, the activity and titer of specific immunoglobulin Y in serum, which were determined by ELISA, showed the presence of antigen specific antibodies for the specific pathogenic bacteria. As others also reported, ELISA is a sensitive technique to find out the antibody titers during different purification processes (15). Further studies have to be conducted to evaluate the potency of these antibodies in treatment of variety of diseases. The outcome of this research work would be an alternative to the current antibiotic treatments. As mentioned earlier, IgY These IgY treatments has a variety of significant like it is safer, more efficient and less expensive as compared to conventional mammalian antibiotics for controlling pathogenic bacteria like *E. coli*. Recently, successful progresses in industrialization of IgY has been achieved in Japan, where IgY as a bioactive ingredient in food, nutraceuticals, cosmetics and other sectors is applied (16). Further studies have to be conducted to evaluate the potency of these antibodies in treatment of variety of diseases. The outcome of this research work would be an alternative to the current antibiotic treatments.

Acknowledgements

The authors are thankful to Karimi aviculture for providing the chickens. Authors also gratefully acknowledged the financial support given by Alborz University of Medical Sciences, Karaj, Iran.

Authors' Contributions

Enayatollah Kalantar, Conceived and designed the experiments, writing the first draft of manuscript, Mohammad Mehdi Soltan Dallal, contributed in literature survey and help in writing the manuscript, Laya Kafami Khorasani, designed preparation of antigen experiments and development of anti-*Escherichia coli* antibodies in chickens, Koursh Kabir, writing the final draft of manuscript, and Monireh Zenolabedini Zamani carried out all the experiments.

Funding/Support

The present study is financially supported by Alborz University of Medical Sciences, Karaj, Iran.

References

- Mahmoudi A, Bakhshi B, Najari-Peerayeh S, Kazemnejad A, Rafeepour Z, Rahbar M. Commensal *E. coli* As An Important Reservoir Of Resistance Encoding Genetic Elements. *Int J Enteric Pathog*. 2014;1(2):44-8.
- Kalantar E, Alikhani MY, Naseri MH, Torabi V. Antibiotic resistance patterns of STEC and ETEC strains: a study on frozen foods of animal origin and children with acute diarrhea. *J Microbiol Infect Dis*. 2013;3(1):31-5.
- Shin JH, Yang M, Nam SW, Kim JT, Myung NH, Bang WG, et al. Use of egg yolk-derived immunoglobulin as an alternative to antibiotic treatment for control of *Helicobacter pylori* infection. *Clin Diagn Lab Immunol*. 2002;9(5):1061-6.
- Chakravarthi PV, Shanmugasundaram K, Malmarugan S. In vitro assessment of bacteriostatic potency of egg yolk immunoglobulin against *Escherichia coli*. *Veterinary World*. 2010;3(10):460-2.
- Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew MJ, et al. Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerg Infect Dis*. 2012;18(5):741-9.
- Collignon P. Resistant *Escherichia coli*—we are what we eat. *Clin Infect Dis*. 2009;49(2):202-4.
- Guimaraes MC, Amaral LG, Rangel LB, Silva IV, Matta CG, Matta MF. Growth inhibition of *Staphylococcus aureus* by chicken egg yolk antibodies. *Arch Immunol Ther Exp (Warsz)*. 2009;57(5):377-82.
- Parma YR, Chacana PA, Lucchesi PMA, Rogé A, Granobles Velandia CV, Krüger A, et al. Detection of Shiga toxin-producing *Escherichia coli* by sandwich enzyme-linked immunosorbent assay using chicken egg yolk IgY antibodies. *Frontiers in Cellular and Infection Microbiology*. 2012;2.
- Kalantar E, Solatni J, Khosravi B, Salehi A. Frequency of *E. coli* pathotypes in acute diarrhea of children and its related factor—Beassat hospital, Sanandaj. *Asian Pac J Tropical Medicine*. 2009;2(4):64-6.
- Subbraj TD, Shanmugam V, Meenatchisundaram S, Michael A, Diraviam T. Isolation, Purification and Neutralizing potential of chicken egg yolk immunoglobulin (IgY) against mastitis causing *Escherichia coli* in dairy cows in Coimbatore District. *Int. J. Drug Dev. & Res*. 2011;3(2):147-53.
- Herbert WJ. Methods for the preparation of water-in-oil and multiple emulsions for use as antigen adjuvants, and notes on their use in immunization procedures. In *Handbook of Experimental Immunology*. 1967:1207-14.
- Sunwoo H, Li X, Lee EN, Kim YK, Sim JS. Egg. Nutrition and Biotechnology. 2000:311-22.
- Sunwoo HH, Lee EN, Menninen K, Suresh MR, Sim JS. Growth inhibitory effect of chicken egg yolk antibody (IgY) on *Escherichia coli* O157:H7. *Journal of food science*. 2002;67(4):1486-94.
- Davalos-Pantoja L, Ortega-Vinuesa JL, Bastos-Gonzalez D, Hidalgo-Alvarez R. A comparative study between the adsorp-

- tion of IgY and IgG on latex particles. *J Biomater Sci Polym Ed.* 2000;**11**(6):657-73.
15. Diraviyam T, Jeevitha T, Saravanan P, Michael A, Meenatchisundaram S. Preparation of chicken (IgY) antibodies consortium for the prevention of enteric infections in poultry. *J. Microbiol. Biotech. Res.* 2011;**1**(4):95-103.
16. Abdou AM, Sato K, Kim M. *Functional proteins and peptides of hen's egg origin.*: Intech Open Access; 2013.