Research article

Production of egg yolk antibody (IgY) against a chimeric protein containing IpaD, StxB, and TolC antigens from Shigella: An investigation of its prophylactic effects against Shiga toxin (Stx) and *Shigella dysenteriae in vitro* and *in vivo*

Nahal Hadi [a], Shahram Nazarian [b,**], Saber Rouhi [c], Seyed Edris Hosseini [c], Javad Fathi [a,*]

[a] *Department of Bacteriology and Virology, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[b] *Department of Biology, Faculty of Basic Science, Imam Hossein University, Tehran, Iran*
[c] *Resident of Large Animal Internal Medicine, Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Iran*

A B S T R A C T

Shigella is a major problem in developing countries. Immunoglobulin Y (IgY) can be used for prophylaxis and neutralize bacteria. The aim of this study was to produce IgY against the chimeric protein containing IpaD, StxB, and TolC antigens from *Shigella*, investigate its prophylactic and neutralizing effects against Stx and *Shigella dysenteriae*. The nucleotide sequence corresponding to the chimeric protein was cloned into pET28a plasmid and expressed in *E. coli* BL21 (DE3). Protein expression was confirmed by SDS-PAGE and the recombinant protein was purified by Ni-NTA affinity chromatography. The 150 μg of chimeric protein was mixed with Freund's adjutant and injected into laying hens (Leghorn). IgY was purified using PEG6000 precipitation. Antibody titer in the serum and egg yolk was evaluated by ELISA. IgY challenge against 1,10 and 50 LD_{50} of Stx and *S. dysenteriae* was investigated. A 60.6 kDa recombinant protein was confirmed by SDS-PAGE. ELISA showed that the antibody titer was significantly increased. MTT assay [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] showed that at 16 μmol/L, IgY protected HeLa cells against Stx. Treatment of mice with 1000 and 1500 μg IgY leads to complete survival of the mice against $1LD_{50}$ toxin and 4000 μg of IgY led to complete survival against $1LD_{50}$, also 70% and 30% survival against 10 and 50 LD_{50} *S. dysenteriae*. This study showed that IgY produced against Stx and *Shigella* virulence factors could cause high protective effects against bacteria and toxins.

1. Introduction

Diarrhoea is an important disease in public health and Enterobacteriaceae are one of the most important causes of diarrhoea. Among the members of this family, Shigella species causes dysentery [1,2]. Factors including unsafe water, raw meat consumption, and contaminated food can lead to the transmission of *Shigella* spp. to humans [3]. According to WHO report, more than 95% of *Shigella*

infections occur in developing countries, with about 80–165 million cases in a year. Also, 11% of deaths of children under 5 years are due to *Shigella* infection and diarrhoea [4]. *Shigella* with its virulence factors can cause hemorrhagic diarrhoea and hemolytic uremia syndrome in the patients and eventually lead to the patient's death [5]. *Shigella* uses the type III secretion system to transfer proteins and toxins to the host cell. Invasion plasmid antigen D (IpaD) plays a role in regulating the type III secretion system and leads to apoptosis in B lymphocytes through interaction with Toll-like receptor 2 (TLR-2) [6,7]. Shiga toxin (Stx) is an AB_5 toxin, and the B subunit of the toxin is attached to the glycosphingolipid globotriaosylceramide (Gb3, CD77) that is present in the intestinal, glomerular, and brain endothelial cells and prepares the conditions for the entry of A subunit to the cells by creating a pore. The A subunit causes cleavage of ribosomal RNA that leads to inhibition of protein synthesis and ribotoxic stress that finally leads to apoptosis [8,9]. TolC protein is a membrane efflux protein (OEP) and plays a role in the release of molecules and toxins from Enterobacteriaceae. This protein leads to bacterial resistance to antibiotics, detergents, dyes, and organic solvents. Therefore, TolC leads to an increase in antibiotic resistance and treatment failure [10]. Invasive mechanisms, resistance to antibacterial agents, increasing antibiotic resistance, as well as the lack of an effective and efficient vaccine against *Shigella* led to the development of new approaches such as prophylaxis with specific antibodies [11–13].

Egg yolk antibody (IgY) is an effective antibody in prophylaxis. The advantages of using IgY compared to mammalian antibodies include non-invasive production, purification by a simple method, cost-effectiveness, and no interaction with complement and mammalian Fc receptors [14–16]. This antibody has high specificity for binding and inactivating antigens and bacteria that enter the host's body [17,18]. An important strategy to prevent the infection and spread of *Shigella* in the host is to inactivate the invasive, secretory, binding and toxin factors using specific IgY before binding and entering the cell [11].

Based on the foregoing, this study aimed to produce egg yolk antibody (IgY) against a chimeric protein containing IpaD, StxB, and TolC antigens from *Shigella*, and investigate its prophylactic effects against Stx and *S. dysenteriae in vitro* and *in vivo*.

2. Material and methods

2.1. Ethical approval

This study was approved by the institutional Ethics Committee of Shiraz University of Medical Sciences (approval No. IR.SUMS. REC.1400.347).

2.2. Expression and purification of the recombinant chimeric protein

A recombinant protein containing epitopes for the IpaD, StxB, and TolC antigens (IST) was expressed and purified according to a previous study [5]. Briefly, the pET28a vector containing IST sequence was transformed into *E. coli* BL21 (DE3) and recombinant protein expression was induced by adding Isopropyl β-d-1-thiogalactopyranoside (IPTG). After confirming the protein expression using the SDS PAGE technique, we purified the protein with the Ni-NTA chromatography column. The concentration of purified recombinant protein was measured by Bradford assay [19].

2.3. Immunization of laying hens with IST antigen

Leghorn-laying hens were used for the production of IgY. Laying hens divided into test and control groups (2 laying hens/group). In the first injection, 150 µg (200 µL) of IST antigen was mixed with an equal volume of Freund's complete adjuvant and it was injected subcutaneously into one site of each laying hen. Booster injections (second and third injections) at two-week intervals with 150 µg of IST antigen and an equal volume of incomplete Freund's adjuvant were given subcutaneously. Also, an equal volume of PBS buffer and adjuvant was injected into the control group. One week after each injection, blood sampling was performed and the serum was stored at −20 °C. From the first injection to two weeks after the last injection, the eggs were collected daily, recording the date and time of injection and kept at 4 °C [13,20].

2.4. Purification of IgY

Purification of IgY was done after the completion of the immunization process of laying hens. Precipitation with polyethylene glycol (PEG6000) method was used for antibody purification [20]. Under sterile conditions, egg yolks were separated and mixed with twice the volume of sterile PBS buffer. 3.5% w/v PEG was added to the egg yolk and buffer and homogenized by shaking. The homogenized mixture was centrifuged at 10,000 rpm for 10 min at 4 °C, and the supernatant was filtered with filter paper. 8% w/v PEG was added and the previous steps were repeated. Finally, 12% w/v PEG was added and after centrifugation, the precipitate was dissolved in 1 ml of sterile PBS buffer and dialyzed against PBS buffer for 24 h. SDS-PAGE 9% was used to check the purity. Also, the concentration of purified IgY was calculated by the Bradford biochemical method [17].

After purification of IgY, Western blot technique was used to determine the reactivity of IgY against IST recombinant protein. SDS-PAGE gel transferred on nitrocellulose membrane under semi-dry conditions and blocking was done using skim milk (5%) plus PBST solution overnight. Then, membrane was incubated in one of the initial antibodies (5 µg/ml of anti-IST IgY) for 2 h. The membrane was incubated with HRP-conjugated rabbit anti-IgY antibody at the dilution level of 1:2000 for 2 h at room temperature. Detection was finally achieved by 3,3′-Diaminobenzidine (DAB) substrate, and the reaction was terminated by deionized water [21].

2.5. Evaluation of antibody titer against IST antigen

Enzyme-linked immunosorbent assay (ELISA) was used to assess the antibody response against IST antigen in the egg yolk and serum [13]. 5 μg (7 μL) per well of IST antigen was mixed in 100 μL coating buffer (64 mM Na_2CO_3, 136 mM $NaHCO_3$, pH 9.8) and added to each well of the ELISA plate. The plate was incubated overnight at 4 °C and washed with Tween buffer (Phosphate Buffered Saline with 0.05% Tween 20 (PBST); the wells were blocked with 100 μL PBST containing 5% dry milk powder and incubated for 1 h at 37 °C. After washing the plate, we prepared the serum and IgY with a dilution of 1:100 to 1:256000 in PBST and added them to the wells; then, we incubated them for 1 h at 37 °C. 100 μL of anti-chicken IgY-HRP conjugate (1:2000) (Sigma Company, United States of America) were prepared and added to the wells and after incubation at 37 °C for 30 min, the plate was washed. 100 μL of substrate solution (1 mg of *o*-phenylenediamine (Sigma), 5 mL citrate buffer (pH 5) and 3 μL H_2O_2) was added to the wells and the plate was kept in a dark place for 10 min. The reaction was stopped by adding 2.5 M H_2SO_4, and finally the absorbance was read at 492 nm. The serum and egg yolk of laying hens in the control group were used as ELISA controls.

2.6. In vitro toxin neutralization assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to investigate the neutralizing effect of IgY against Stx. At the first, Purification of Stx toxin was performed according to a previous study [22]. A 50% cytotoxic dose (CD_{50}) of Stx toxin was calculated. The serial dilutions of Stx were prepared in PBS buffer (2.5–0.00125 ng) and added to the wells containing HeLa cells and 0.5% Dulbecco's modified Eagle's medium (0.5% DMSO) with 10% fetal bovine serum. The plate was incubated for 12 h at 37 °C and 5% CO2. After incubation, the supernatant was removed and a new medium (containing 0.5 mg/mL MTT) was added to the wells and incubated for 4 h under the previous conditions. Finally, the supernatant was removed and dimethyl sulfoxide was added to the wells and after incubation for 15 min, the absorbance was read at 590 nm and CD_{50} Stx was evaluated [23]. Different concentrations (0–20 μmol/L) of IgY were prepared and added to the plates containing HeLa cells with CD_{50} toxin stx and incubated at 37 °C and 5% of CO_2 overnight. The 50% inhibitory concentration (IC_{50}) of IgY was determined.

2.7. In vivo toxin neutralization assay

The toxin neutralization challenge by IgY was performed in BALB/c female mice aged six to eight weeks with an approximate weight of 20–30 g (purchased from the Pasteur Institute, Iran). Before the challenge, 50% lethal dose (LD_{50}) of Stx toxin was calculated by the method of Reed and Muench [24]. Briefly, 10-fold serial dilutions of Stx were prepared in sterile PBS buffer and injected intraperitoneal into 5 groups of BALB/c mice (6 mice/group) (replicated three times). The survival and mortality rates of mice were checked and recorded for 7 days. After determining the LD_{50}, we mixed the concentrations of 1 (0.25 ng/μl), 10 (2.5 ng/μl) and 50 (12.5 ng/μl) LD_{50} of Stx with concentrations of 250 μg (30 μl), 500 μg (60 μl), 1000 μg (120 μl) and 1500 μg (176 μl) of IgY and after 1 h of incubation at 4 °C, they were injected (intraperitoneal) into the groups of mice (10 mice/group). The survival rate of mice was compared to the group receiving $1LD_{50}$ of Stx [25].

2.8. In vivo bacterial challenge

First, the LD_{50} of *S. dysenteriae* was calculated. The bacteria were cultured in Tryptic Soy Broth (TSB) medium. At $OD_{600} = 1$ (After one overnight (18 h) and log phase growth of *S. dysenteriae*), the culture media was centrifuged, and serial dilutions (1×10^9 to 1×10^5 colony forming unit (CFU) were prepared from the bacterial sediment in PBS buffer and injected intraperitoneally into the groups of BALB/c female mice (6 mice/group) [26]. Concentrations of 1, 10 and 50 times LD_{50} were mixed with 1000 μg (120 μl), 2000 μg (235 μl) and 4000 μg (470 μl) μg of IgY and incubated at 4 °C for 1 h. This mixture was injected intraperitoneally into the groups of mice (10 mice/group). The survival rate of mice was compared to the group which received $1LD_{50}$ of *S. dysenteriae*.

2.9. Statistical analysis

Statistical analyses were carried out using SPSS 22.0. Kolmogorov- Smirnov test was used to test the normality of the data. *t*-test was used to comparison between the control and test groups. For tests, p-value less than 0.05 was considered significant.

3. Results

3.1. Expression and purification of the recombinant chimeric protein

According to a previous study, by transforming the pET28a vector containing IST sequence into *E. coli* BL21 (DE3), recombinant IST protein was expressed. The presence of IST with a molecular weight of 60.6 kDa was confirmed by the SDS-PAGE technique. Protein purification was done with Ni-NTA chromatography column and under denaturing conditions (Imidazole 250) [4]. The final concentration of purified protein was approximately 750 μg/ml.

3.2. Evaluation of antibody titer against IST antigen

Evaluation of the immune response in the serum (Fig. 1) and egg yolk (Fig. 2) showed that the injection of IST antigen in laying hens led to a significant increase in their antibody titer in booster injections, as compared to the control group.

The results of serum ELISA of immunized laying hens compared to the control and booster doses showed a significant difference in the increase of antibody titer (P < 0.01), and in 1:100 dilution of the serum, the optical density (OD) increased from 1.2 to 2.9.

As shown in Fig. 2, the amount of antibody production in egg yolk compared to the control and booster doses showed a significant difference in the increase of antibody titer (P < 0.01), and in 1:100 dilution of the purified antibodies, the OD increased from 2.11 to 3.55.

3.3. Purification and confirmation of IgY

After ELISA and showing that the antibody response increased between the second and third doses, it was decided to purify of IgY after the third injection. Purification of IgY was done by polyethylene glycol precipitation method and the quality of the purified antibody was checked by 9% SDS-PAGE (Fig. 3A).

Gel staining and examination of protein bands showed that the method used to purify IgY was an efficient one, and the IgY in full form and its light and heavy chains were located in the expected position. The measurement of IgY concentration showed that the concentration of purified IgY was 8.5 mg/ml. Also, western blotting analysis using HRP-conjugated rabbit anti-IgY antibody showed that the reactivity of the IgY was done with the IST recombinant protein (Fig. 3B).

3.4. Identification and binding of IgY to IST antigen

ELISA method was used to determine the minimum amount of purified IgY capable of recognizing and binding to IST antigen (Fig. 4). This Figure shows that the minimum IgY antibody concentration in the detection range of the linear that reacted with the antigen in the ELISA method was 39 ng.

3.5. In vitro toxin neutralization assay

The result of IgY cytotoxicity neutralization assay against Stx toxin is presented in Fig. 5.

Based on these results, the cell survival rate is highly dependent on the concentration of the IgY. A concentration of 16 μmol/L of IgY caused complete protection of cells against 5 CD_{50} Stx toxin.

3.6. In vivo toxin neutralization assay

Challenge with Stx and IgY showed that in the group receiving the only Stx, all mice died after 5 days. In the group that received IgY control laying hens and Stx, all the mice died. The survival results of mice that received 1, 10 and 50 times LD_{50} of Stx toxin and different concentrations of anti- IST IgY are shown in Fig. 6. The results of mice groups that received 1, 10 and 50 times LD_{50} of Stx toxin and 250 μg (30 μl) of anti- IST IgY are shown in Fig. 6A. According to this Figure, mice that received 1 LD_{50} of Stx and 250 μg of
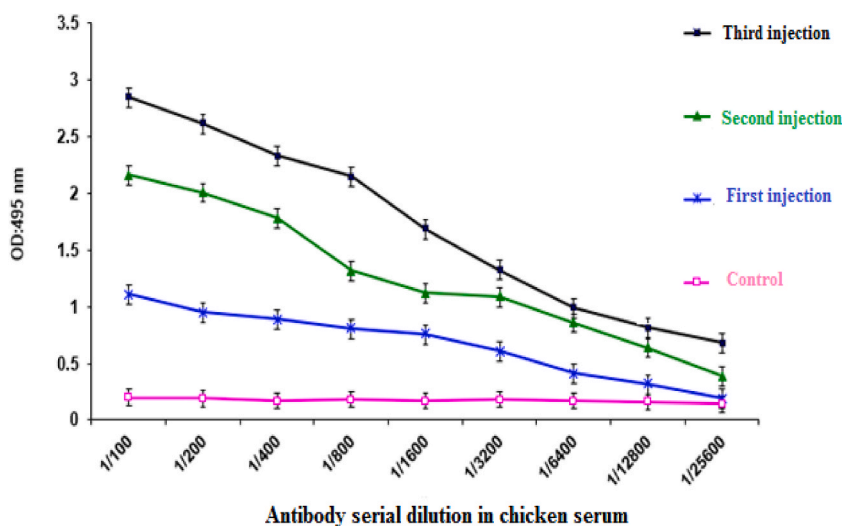

Fig. 1. Antibody titer analysis in the serum (sera was taken one week after each injection – weeks 1, 3 and 5) of laying hens immunized with IST antigen and Freund's adjuvant compared to the control group (PBS with Freund's adjuvant (P < 0.01).
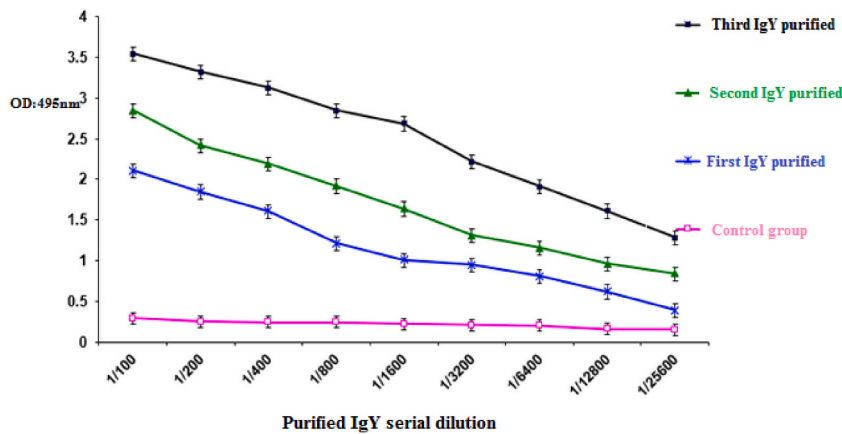
Fig. 2. Antibody titer analysis in the egg yolk (was taken one week after each injection, weeks 1, 3 and 5) of laying hens immunized with IST antigen and Freund's adjuvant compared to the control group (PBS with Freund's adjuvant) (P < 0.01).
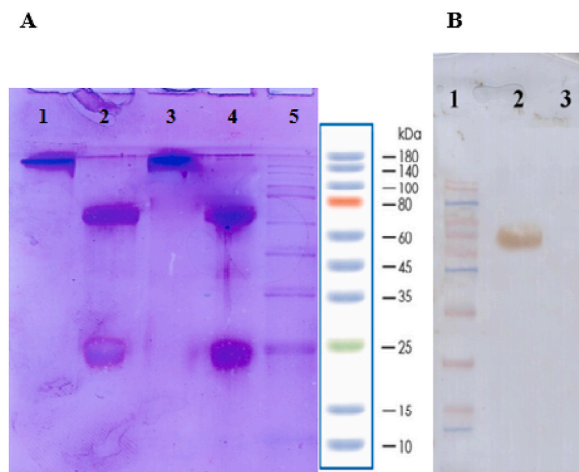

Fig. 3. A: The SDS-PAGE of IgY purification from the egg by PEG method (supplementary file): column 1 and 3 - antibody sample electrophoresed in non-reducing conditions (180 kDa) (without 2-mercaptoethanol), column 2 and 4 - antibody electrophoresed in reducing conditions (with 2-mercaptoethanol) and column 5- protein marker ((10-180 KDa, SinaClone, Iran).
B: Western blot analysis of IST protein using purified IgY (supplementary file). Lane 1: Protein marker (Vivantis Prestained Protein Ladder: PR0602), Lane 2: recombinant IST protein, Lane 3: BSA as negative control.

IgY had 20% survival, while the groups that received 10 and 50 LD_{50} of Stx and 250 μg of IgY, died on the seventh day (two days longer than the control group).

The groups that received 500 μg (60 μl) of IgY are shown in Fig. 6B; in the group that received 1 LD_{50} of the Stx toxin, 50% survival was observed, and in the group that received 10 LD_{50} of the toxin, the survival rate was 20%; also, the group which received 50 LD_{50} died on the seventh day (two days longer than the control group). Fig. 6C demonstrates the groups that received 1000 μg (120 μl) of IgY and 1, 10 and 50 times LD_{50} of Stx toxin. The group that received 1 LD_{50} of the Stx toxin had 100% survival; also, in the group which received 10 and 50 LD_{50} of the Stx toxin, the survival was 50% and 20%, respectively.

Also, the group that received 1500 μg (176 μl) of IgY is shown in Fig. 6D. These results showed that 100% survival was observed in the group that received 1 LD_{50} of the Stx toxin; in the group that received 10 LD_{50} and 50 LD_{50} of the Stx toxin, the survival rates were reported 70% and 30%, respectively.

The results of the IgY and Stx toxin challenge show that with increasing antibody concentration, the survival rate of mice against Stx toxin increases significantly.

3.7. In vivo bacterial challenge (IgY- S. dysenteriae)

The intraperitoneal LD_{50} of *S. dysenteriae* was 1×10^7 CFU. The control group received 1 LD50 (1×10^7 CFU) of bacteria and at least half of the mice died after 4 days. The survival results of the mice which received different antibody concentrations and different LD_{50}
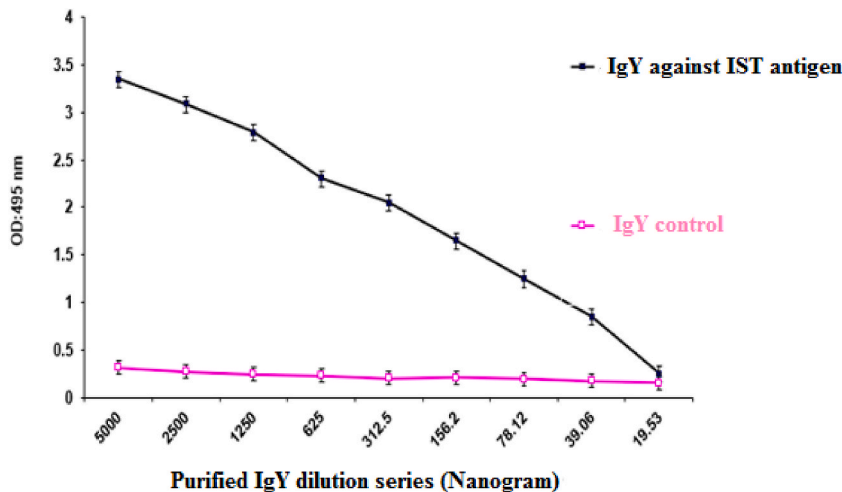
Fig. 4. Determining the minimum amount of purified IgY capable of recognizing and binding to IST antigen. The minimum IgY concentration in the detection range of the linear was 39 ng.
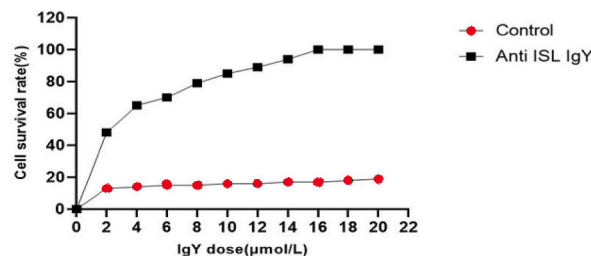

Fig. 5. Cytotoxicity neutralization of anti- IST IgY against 5 CD_{50} Stx toxin in HeLa cells by MTT assay, compared with 20 umole/L IgY purified from the control group (PBS with Freund's adjuvant).

of *S. dysenteriae* are shown in Fig. 7.

Fig. 7A shows the groups that received 1000 μg (120 μl) of IgY and 1, 10 and 50 times LD_{50} of *S. dysenteriae*. As shown in this Figure, the survival rates of the groups that received 1000 μg of IgY and 1, 10, and 50 times LD50 of *S. dysenteriae* were reported 50%, 30% and 10%, respectively.

Fig. 7B shows the groups that received 2000 μg (236 μl) of IgY. In the groups that received 1 LD_{50}, 10 LD_{50}, and 50 LD_{50} of *S. dysenteriae*, the survival rate was reported 70%, 50% and 20%, respectively.

The survival rate of the groups which received 4000 μg (472 μl) of IgY is shown in Fig. 7C.

As shown, the group that received 1 LD_{50} of *S. dysenteriae* survived completely; in the group that received 10 LD_{50} of *S. dysenteriae*, the survival rate was 70%. Also, the group that received 50 LD_{50}, the survival rate was 30%.

In the IgY- *S. dysenteriae* challenge, as well as the IgY-Stx toxin challenge, it was shown that the survival rate of mice against *S. dysenteriae* increased with the increase of the IgY concentration.

4. Discussion

Shigella is the cause of bacillary dysentery and shigellosis and is one of the most contagious bacteria causing diarrhoea, especially in developing countries and countries with low levels of hygiene. *Shigella* is transmitted through water, food, and contaminated vegetables and can threaten the lives of people infected with this bacteria, especially children [27–29]. Although many studies and efforts have been made by modern medicine and public health to control and prevent the spread of *Shigella*, so far these efforts have not had acceptable and effective results [30]. *Shigella* is an intracellular organism, so it escapes from the host's immune system. Today, the frequency of antibiotic resistant *Shigella* strains is increasing, and these strains show resistance against fluoroquinolones, cephalosporins, and azithromycin [31]. Outer membrane proteins, surface proteins, and secreted toxins of bacteria are considered good candidates for inducing antibody production because they are directly accessible to the B and T cells of the host's immune system [32]. Due to the increasing antibiotic resistance and the lack of an effective vaccine against *Shigella* strains, it seems that the use of antibodies, as an alternative for prevention and prophylaxis, is an effective strategy to control infection and disease. Based on the results of the previous research and the confirmation of the function of the IgY antibody produced by actively immunizing chickens in neutralizing *Shigella*, it seems that this protein can be used for the production of chicken antibody in order to create passive immunity
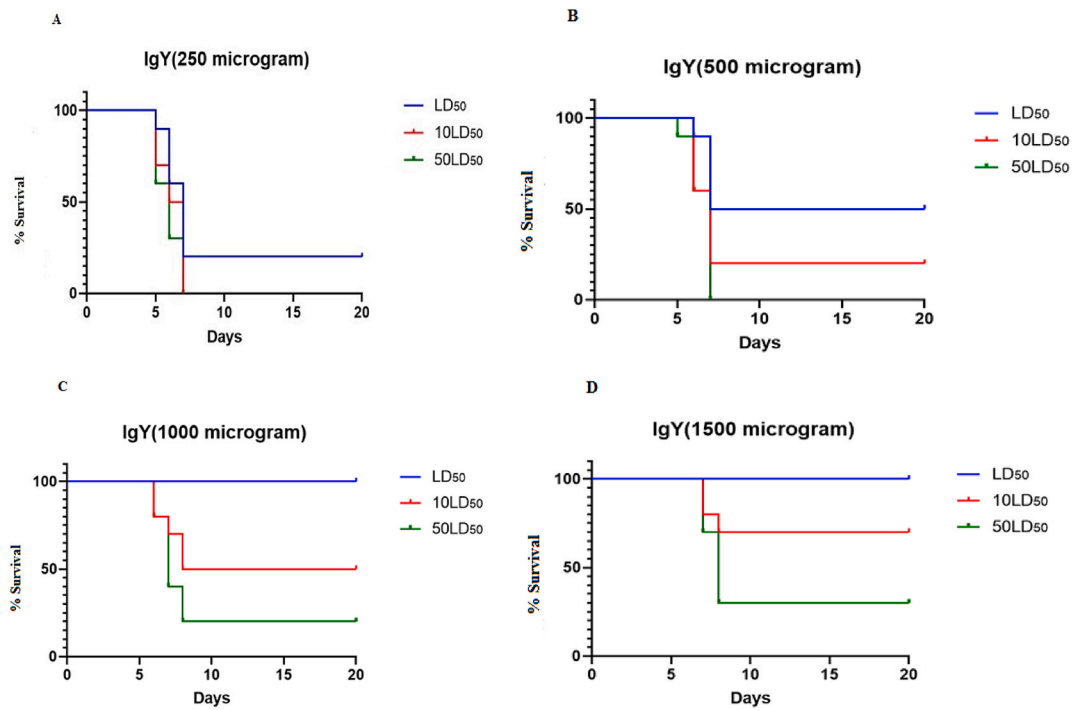
Fig. 6. Comparison between groups of mice (10 mice/group) that received 1, 10, and 50 times the LD50 of Stx and 250 μg (A),500 μg (B),1000 μg (C) and 1500 μg (D) of anti-IST IgY compared to the control group (1500 μg IgY purified from laying hens receiving PBS with Freund's adjuvant).
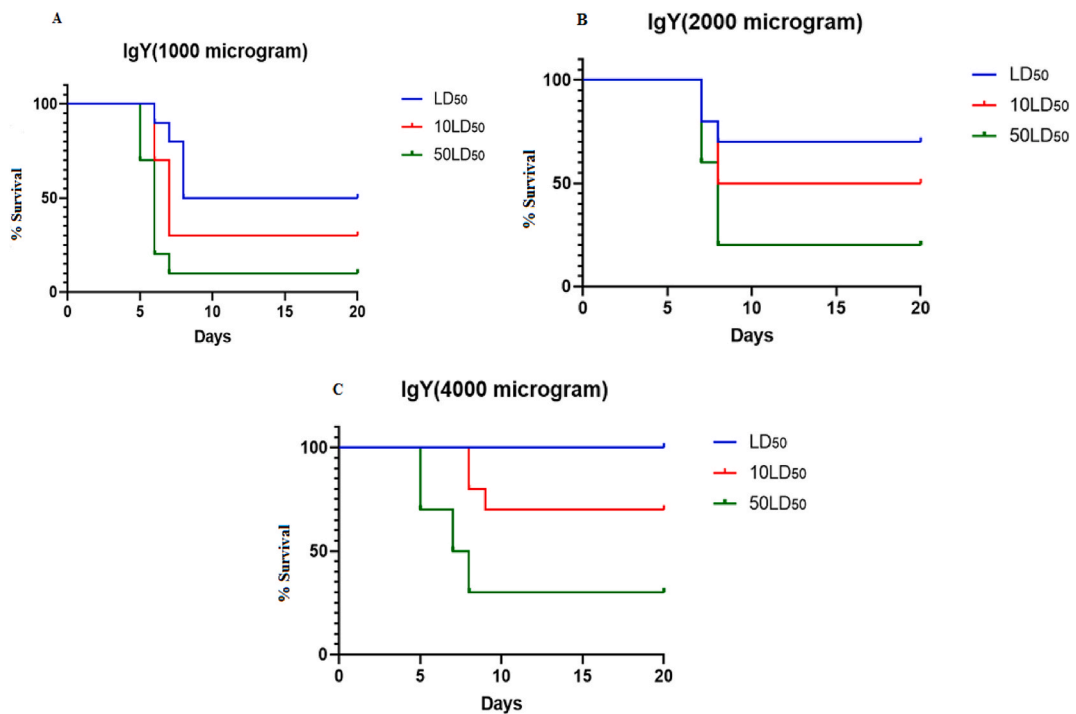
Fig. 7. Comparison between groups of mice (10 mice/group) that received 1, 10 and 50 times LD50 of *S. dysenteriae* and 1000 μg (A), 2000 μg (B) and 4000 μg (C) of anti- IST IgY compared to the control group (4000 μg IgY purified from laying hens receiving PBS with Freund's adjuvant).

against *Shigella*. Egg yolk antibody (IgY) is a suitable candidate for prophylaxis due to its cost-effectiveness and easier production and purification than mammalian IgG [32–35]. Based on a previous study, it was determined that the chimeric protein consisting of IpaD, StxB, and TolC (IST) of *Shigella* spp. had the ability to stimulate the immune system and produce antibodies [5,26]. In this study, after expressing and purifying IST antigen and injecting it into laying hens, the amount of purified IgY against IST recombinant protein was 8.5 mg/ml (after third administration the OD = 3.6). In the study of Fathi et al., the amount of IgY produced against Stx toxin was approximately 5 mg/ml (after third administration the OD = 1.9) [13]. A 1.7 times more than concentration of IgY in the present study compared to the study of Fathi et al. may be due to the use of chimeric protein containing three antigens for immunization, while in the study of Fathi et al. only Stx toxin was used. In Parma et al.'s study, the production of IgY anti-StxB subunit was done and yield of purified of IgY was 0.84 mg/ml [25]. Based on previous studies that used an antigen to stimulate antibody production, can be expected that the reason for the low IgY value is the use of only B subunit toxin for immunization. Maybe, the high level of IgY production in the present study can confirm that multivalent chimeric proteins have a good ability to induce the host's immune system. In the present study, it was found that the minimum antibody concentration in the detection range of the assay that reacted with the antigen in the ELISA method was 39 ng, this amount in Parma et al.'s study for purified wild-type Stx2 was 115 ng [36]. In Wang et al.'s study, the concentration of 17.8 μmol/L of IgY caused complete protection against Stx toxin in the cell line [37]. The result of Wang et al.'s study was similar to that of the present study, which required a concentration of 16 μmol/L of IgY to protect the cells against Stx toxin. In the present study, *in vivo* toxin neutralization assay showed that concentrations of 250 μg (30 μl) and 500 μg (60 μl) of IgY were able to provide acceptable protection at 1 LD_{50} from Stx toxin, and at 10 LD_{50} and 50 LD_{50}, could increase the survival time of mice compared to the control group. However, the concentrations of 1000 μg (120 μl) and 1500 μg (176 μl) of IgY caused 100% protection of mice against 1 LD_{50} of Stx toxin, and the concentration of 1500 μg (176 μl) of IgY caused 70% and 30% protection against 10 LD_{50} and 50 LD_{50} of Stx toxin, respectively. In the study carried out by Fathi et al., concentrations of 500 and 1000 μg of IgY provided 40% and 60% protection against the 5LD_{50} of the toxin, respectively, and the concentration of 2000 μg completely protected the mice against the toxin. It should be mentioned that in the study of Fathi et al. only 5LD_{50} of toxin was used, while in the present study, 10 and 50LD_{50} were used; also, they used the complete Stx protein for immunogenicity, but in this study, the B subunit of Stx toxin was used combined with two other antigens. In another study, Wang et al. produced IgY against StxB. The results of the Stx-IgY challenge showed that concentrations of 1200 and 3600 μg of IgY caused 40% and 100% protection of mice against 5LD_{50} of Stx toxin, respectively [37]. Although in the study of Wang et al., the complete protection against the toxin was at a concentration of 3600 μg of IgY and 5LD_{50}, in our study, complete protection was observed at a concentration of 1000 μg (120 μl) and 1500 μg (176 μl) of IgY against 1LD_{50} and also 20% and 30% protection against 10 and 50 LD_{50}, respectively. Also, in *in vivo* bacterial challenge, it was found that IgY increased the survival of mice against *S. dysenteriae*. A concentration of 4000 μg (470 μl) of IgY caused 100% survival against 1 LD_{50} of *S. dysenteriae*. Also, this concentration of IgY caused 70% and 30% survival of mice against 10 and 50 LD_{50} against *S. dysenteriae*, respectively. The results of this study showed well that IgY could be used as an effective and efficient method against infections and reduced disease symptoms and subsequent mortality.

In conclusion, diarrhoea caused by *Shigella* is a concern in developing countries. Increasing antibiotic resistance and lack of approved vaccine for *Shigella* strains led to the development of prevention methods such as prophylaxis. The results of the study and previous studies show that IgY may be able to be used as a prophylaxis method to prevent *S. dysenteriae* infection.

Funding statement

Data availability statement

All data is provided in this article.

CRediT authorship contribution statement

Nahal Hadi: Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shahram Nazarian:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation. **Saber Rouhi:** Writing – review & editing, Writing – original draft, Methodology. **Seyed Edris Hosseini:** Writing – review & editing, Methodology. **Javad Fathi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Javad Fathi reports financial support and equipment, drugs, or supplies were provided by Shiraz University of Medical Sciences. Javad Fathi reports a relationship with Shiraz University of Medical Sciences that includes: board membership and employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Abbreviations

IgY: Immunoglobulin Y
TLR-2: Toll-like receptor 2
IpaD: Invasion plasmid antigens D
IPTG: Isopropyl β-d-1-thiogalactopyranoside
ELISA: Enzyme-linked immunosorbent assay
LD_{50}: 50% lethal dose
TSB: Tryptic Soy Broth
CFU: Colony Forming Unit
OD: Optical Density