Production of specific IgY antibody to the recombinant FanC protein produced in *Escherichia coli*

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**ABSTRACT**

**Objective(s):** Enterotoxigenic *Escherichia coli* (ETEC) strains are one of the primary causes of diarrhea in newborn calves and in humans, pigs, and sheep. IgY technology has been identified as a promising alternative to generating a mass amount of specific antibody for use in immunotherapy and immunodiagnostics. The purpose of this study was to produce specific antibody by egg yolk antibody (IgY) to recombinant FanC protein from *ETEC*.

**Materials and Methods:** FanC (K99) gene was amplified from *ETEC* by specific primers and polymerase chain reaction. The gene was cloned and subcloned into pT7Z/R/T and pET32a (+) vectors, respectively. Recombinant vector was transferred into *E. coli* BL21 CodonPlus (DE3). Protein expression was investigated by 1 mM IPTG induction. Hens were immunized by the purified recombinant FanC protein. The activity and specificity of the IgY antibody were detected by dot-blotting, Western blotting, and indirect ELISA.

**Results:** We obtained FanC specific IgYs by immunizing the hens with the recombinant FanC protein. The anti-FanC IgY showed binding specifically to the FanC protein of *ETEC*.

**Conclusion:** The results emphasize that specific IgY against the recombinant FanC protein could be recommended as a candidate for passive immunization against *ETEC* infection in animals and humans.

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**Introduction**

*Enterotoxigenic Escherichia coli* (ETEC) strains are the major cause of diarrhea (1). *ETEC* expressing K99 fimbria is a bacterium that causes diarrhea in calves, lambs, piglets (2), and humans (3) resulting in mortality, morbidity, reduction of live weight, and huge economic losses (4). The K99 protein located at the surface of *ETEC* is a polymeric protein structure and has a diameter of 5 nm (2). K99 fragment encodes eight gene products named FanA to FanH, all of which are required for biosynthesis of K99 (5). The nucleotide sequence of the FanC gene comprises 159 amino acids which are proceeded by the signal sequence of 22 residues (6).

Chicken egg yolk (IgY) has been used widely for treatment and prevention of infections in humans and animals (7). It is used for passive protection against pathogen infections such as *Bovine rotavirus*, *ETEC*, *Bovine coronavirus*, *Salmonella*, *Staphylococcus* and *Pseudomonas* (8). Passive immunization using oral administration of specific antibodies such as IgY represents an effective strategy to prevent gastrointestinal infection in animals (9). The present study was carried out to characterize the specific IgY antibody produced by immunizing the hens to the recombinant FanC protein expressed in *E. coli*.

**Materials and Methods**

**PCR amplification**

A strain of Iranian *ETEC* was isolated from diarrhea samples of newborn calves that was positive for the K99 antigen. It was provided by Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran, and confirmed by biochemical and molecular tests. Genomic DNA of *ETEC* was extracted by using a DNA extraction kit (Bioneer, Korea). One pair of specific primer was designed and synthesized by Macrogen (South Korea) as follows: FanC-Forward: 5'–*CGCGGAT CTGCC-GCCGAATACAGGTACTATTACAATTT-3'* and FanC-GC-Reverse: 5'–*CGCCGAT CGCC-GCCGAATACAGGTACTATTACAATTT-3'*

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Reverse: 5'-CCGCTCGAGCAGAGCATATAAGTGAATACAAGAGATG-3' (492 bp). BamHI and XhoI restriction sites were placed for gene transfer FanC to expression vector in forward and reverse primers, respectively (underlined). Polymerase chain reaction (PCR) was carried out in total volume of 25 μl containing 1.5 μl of MgCl₂ (50 mM), 2.5 μl of 10X PCR buffer, 2 μl of dNTPs (20 mM), 2 μl of mix primer (5 pmol/μl), 1 μl of DNA template (50 to 100 ng/μl) and 0.2 U/μl of ExTaq DNA polymerase, and 15.8 μl of deionized water. The PCR cycle conditions were an initial denaturation at 94 °C for 10 min, followed by 34 cycles of 94 °C for 30 sec, 48 °C for 30 sec, 72 °C for 30 sec, and final extension at 72 °C for 10 min. Amplified PCR fragment was separated by electrophoresis in a 1% agarose gel, stained with ethidium bromide, visualized under UV light and photographed with a UVidoc GEL Documentation System (UVitec, UK).

Cloning and sub-cloning the FanC (K99) gene

The purified PCR product by GeneJET Gel Extraction Kit (Fermentas) was ligated into pTZ57R/T cloning vector by T/A cloning. The recombinant vectors were transformed into competent E. coli DH5α. The bacterial clones harboring recombinant plasmid DNA were screened based on their ampicillin resistance. The recombinant vector was investigated by PCR reaction using FanC specific primers and M13F (5'-TGTAAAACGACGGCCAGT-3') and M13R (5'-CAGGAAACAGCTATGACC-3') primers. The PCR product was visualized by 1% agarose electrophoresis.

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Expression and protein purification of FaC gene

E. coli BL21 CordonPlus (DE3) harboring the FanC expression construct was grown in Luria broth (LB) culture supplemented with 100 μg/ml ampicillin and incubated overnight at 37 °C and 150 rpm. Fresh LB liquid (50 ml) containing 100 μg/ml ampicillin was incubated with 5 ml of preculture and was incubated at 37 °C and 150 rpm to reach OD₆₀₀ of 0.6. Then, the culture was induced with 1 mM IPTG and incubated at 37 °C with shaking at 150 rpm for 6 hr. Cells were harvested at different time points after induction. The FanC expression was evaluated on 12% SDS-PAGE and visualized using Coomassie-blue staining.

For purification, the pellet of the induced cells was resuspended in lysis buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM Imidazole, pH=8). Lysozyme was added in concentration of 1 mg/ml. It was incubated on ice for 30 min. The cells were disrupted by sonication. Subsequently 1 ml of the 50% Ni-NTA (Qiagen, USA) slurry was added to 10 ml cleared lysate and was mixed by shaking at 4 °C for 60 min. The lysate-Ni-NTA mixture was load into a column with the bottom outlet capped. Then, the recombinant protein was washed once with 4 ml washing buffer (50 mM NaH₂PO₄, 300 mM NaCl, 20 mM Imidazole, pH=8). The 6xHis-tagged recombinant protein was eluted 4 times with 0.5 ml elution buffer (50 mM NaH₂PO₄, 300 mM NaCl, 250 mM Imidazole, pH=8) per column each time and was collected in 4 tubes. The concentration of the recombinant FanC protein was determined by Bradford assay (10). Before the injection of the recombinant FanC protein to the hens, the recombinant FanC protein was dialyzed to remove imidazole and other impurities.

Dot-blotting analysis of the recombinant FanC protein

The recombinant FanC protein (2 μg) was induced with 1 mM IPTG, the extract of the transformed bacteria unincluded by IPTG and PBS were dot-blotted on nitrocellulose membrane. The membrane was immersed in 1% bovine serum albumin (BSA) and was shaken for 30 min at room temperature, it was washed with PBST (PBS, 0.1% (v/v) Tween) for 2 min, then it was immersed in primary antibody (Anti-His tag rabbit) diluted 1:500 for 1 hr on a shaking incubator at room temperature. Afterward, the nitrocellulose membrane was washed 4 times for 5 min each time in PBST, it was incubated with a secondary antibody that was diluted 1:3000 for 1 hr on a shaking incubator at room temperature. The membrane was washed 4 times for 5 min each time in PBST. Color development was observed by addition of dianinobenzidine dissolved in PBS and H₂O₂.

Immunization

Immunization of the hens was performed by intramuscularly injecting with 200 μg of purified protein with an equal volume of Freund's complete adjuvant (Sigma, USA) for the first immunization. Freund’s complete adjuvant without recombinant protein was injected to the control group. Two booster immunizations followed using incomplete Freund’s adjuvant with two weeks interval. One week after the last of injection, the eggs were collected daily for eight weeks, marked and stored at 4 °C.
**IgY antibody purification**

IgY antibody was extracted and purified from egg yolk using polyethylene glycol 6000 (PEG 6000) powder (Merck, Germany) based on Polson's method (11). Egg yolk was separated from the egg, Phosphate-buffered saline (pH=7.6, twice the volume of yolk suspension) was added and mixed. The PEG 3.5% (w/v) was added and then shaken at room temperature for 10 min followed by centrifugation (13000 g, 20 min, 4 °C). The supernatant was collected and filtered through a Whatman filter paper to remove lipids. PEG 6000 was added to 8% (w/v) concentration, after gentle shaking at the room temperature for 10 min, again it was centrifuged (13000 g, 20 min, 4 °C). The precipitation was dissolved in 10 ml PBS and then PEG 6000 was added and centrifuged as described above. The pellet containing IgY was resuspended in 2 ml PBS and dialyzed against PBS overnight at 4 °C. Specific IgY anti-FanC content was measured by the Coomassie-blue method according to Bradford (10).

**Dot-blotting and Western-blotting of anti-FanC IgY**

2 µg of the recombinant protein was dot-blotted individually on two pieces of nitrocellulose membranes. After similar steps that were mentioned previously, each piece was immersed in primary antibody (hen anti-FanC IgY of the treatment group after injection and hen IgY of the control group) diluted 1:2000 and followed with secondary antibody (rabbit anti-chicken IgY H&L (HRP)) (Abcam, USA) diluted 1:2000. Other steps were carried similar to steps mentioned previously.

The purified recombinant protein was separated using 12% SDS-PAGE and transferred onto nitrocellulose membrane. Sites were blocked with BSA 1.5% (w/v) and incubated at 4 °C overnight. The membrane was incubated with anti-FanC IgY antibody (1:10000) for 1 hr at room temperature. After washing, rabbit anti-chicken IgY H&L (HRP) diluted in PBS (1:2000) was added and incubated for 1 hr. The membrane was washed and the specific binding of IgY antibody to the FanC protein was investigated.

**Indirect enzyme-linked immune sorbent assay (iELISA)**

The specific activity of IgY antibody against the FanC protein was determined by iELISA. 100 µl of the recombinant protein was coated onto microtiter plates (65, 44, 33, and 26 ng) for 1 hr. Plates were washed three times with PBS containing 0.05% (v/v) Tween 20 (PBST) and nonspecific sites were blocked using blocking buffer (1.5% BSA in PBST) for 1 hr at 37 °C. After washing three times, 100 µl of diluted IgY antibody (1:2000) in PBST was added to the wells as the primary antibody and then incubated for 1 hr at 37 °C. Subsequently, the plate was washed again and incubated with 100 µl of labeled rabbit anti-chicken IgY H&L (HRP) at a diluted IgY antibody (1:10000) as the secondary antibody for 1 hr at 37 °C. The colorimetric detection was carried out using O-phenylenediamine (OPD, Sigma) as a chromogenic substrate of HRP and then wells were washed with PBST. The reaction was stopped by adding 50 µl/well 2N H₂SO₄. The absorbance of each well was measured at 492 nm.

**Statistical analysis**

The t-test was used to detect significant differences between means of ELISA absorbance for determination of specific IgY antibody. Also, a repeated measure ANOVA was used to compare the means of ELISA absorbances between weeks. P-value<0.05 was considered significant.

**Results**

**PCR amplification, cloning and sub-cloning of the FanC gene**

The FanC PCR product yielded a specific fragment 492 bp. The PCR amplified product was successfully cloned into pTZ57R/T after being extracted from the agarose gel. The results of PCR assay with specific primers, M13 primers (Figure 1a), and enzyme digestion showed that the target gene was correctly inserted into the recombinant pTZ57R/T plasmid (Figure 1b). A new sequence for the first time has been published on NCBI gene database under accession number KP05429.
Specific IgY antibody to recombinant FanC protein

The results colonies of the recombinant pET32a (+)-FanC plasmid were confirmed by colony PCR with pET T7 primers (Figure 2a). The existence of FanC gene into pET32a (+) was verified by enzymatic digestion (Figure 2b).

Expression and purification of the recombinant FanC protein

The results of the FanC expression were shown on 12% SDS-PAGE electrophoresis by induction on 1 mM IPTG in 2, 4 and 6 hr after induction (Figure 3a). Figure 3 shows that the FanC antigen was highly expressed in E. coli BL21 CodonPlus. The recombinant FanC protein could be detected near 36 kDa (pET32a (+)+FanC). Also, Figure 3 shows proteins near 18.5 kDa that show pET32a (+) without a fragment of the FanC gene. pET32a (+) vector is able to express a fusion protein with a 6-histidine tag at thrombin site and a T7 tag at the N-terminus. These additional amino acids increased the size of the expressed FanC protein near 18.5 kDa.

Immunization and IgY purification

There was no decline in egg laying capacity after the immunization (P-value<0.05); the hens produced an average of one egg per day. The results showed that the combination antigen to Freund’s adjuvant caused neither laying decrease nor adverse effects in the hens. IgY antibody was successfully extracted and purified from the egg yolk by PEG 6000. The purified IgY electrophoresed on SDS-PAGE using 12%, showed that IgY contained two major protein light (23 kDa) and heavy (68 kDa) chains (Figure 4b). In addition, some bands were presented around 40 kDa on the gel, probably due to the C-terminal fragment of the vitellogenin II precursor (12).

Dot blotting and western blotting analyses

Dot-blot analysis of the recombinant FanC protein showed that it could be recognized by the anti-FanC specific IgY in 1:2000 dilution of antibody. Western blot analysis indicated a 36 kDa protein band.

Indirect ELISA of anti-FanC IgY

The anti-FanC antibody activity was measured from ELISA absorbance data. ELISA assay (Figure 5a) indicated positive binding of IgY to FanC as expected. Also, Figure 5a shows that anti-FanC IgY (at 1:2000 dilution) was able to recognize the recombinant FanC protein in the treatment group (immunized by FanC) and there were significant differences between treatment and control groups (P-value<0.0001). These results indicated that FanC is highly immunogenic for chickens. The production of specific IgY from the first to eight weeks after the last
immunization is shown in Figure 5b. The results indicated that high IgY level started to increase two weeks after the complete immunization (P-value<0.05). There was a significant increase of IgY production after the first week and the results showed specific antibody activity was increased (P-value<0.05). Also, significant differences were seen between the second week and other weeks. No significant difference was seen in specific IgY production from the third week to the eighth, indicating that the antibody level remained constant.

**Discussion**

Despite the improvement in herd management, nutrition, and facilities in animal husbandry, diarrhea in calves is still the main problem in this industry. Maternal antibodies (colostrum) protect the neonatal offspring against *ETEC* caused diarrhea in the first days of life (13). Moreover, milk antibodies drop rapidly in cattle that often develop diarrhea after neonatal calves become infected with *ETEC* (14). The development of vaccines or administration of milk supplements that make passive protection for the first several days of life induces a high protection against *ETEC* (15). IgY as an efficient biological agent has shown a significant efficacy in prevention of infectious diseases. The evidences of IgY efficacy reported in several studies.

These studies are including specific egg yolk antibody against recombinant *Cryptosporidium parvum* P23 protein (16), generation of egg yolk antibodies against influenza M2 protein (17), production of IgY against recombinant *Porcine epidemic diarrhea virus* COE protein (18) and production of specific IgY against *Helicobacter pylori* recombinant (19).

Researchers (20) indicated that passive protection of neonatal piglets achieved against K88, K99 and 987P fimbrial adhesions of *ETEC* with specific egg yolk immunoglobulin, and their results of scanning electron microscopy revealed resistance to bacterial adhesion in piglets with high-titer antibodies. In another study (21) IgY was applied for the protection of neonatal piglets from diarrhea caused by *E. coli* (*ETEC*). Malekan et al (22) produced anti-*E. coli* k99 IgY and anti-*Salmonella* IgY by immunizing laying hens with inactivated *E. coli* K99 and *Salmonella enteritidis* antigens. They indicated that immune eggs against *E. coli* k99 and *Salmonella* can be used to stimulate the immune system in newborn calves and protect them against the deleterious effects of these organisms. Hashish et al (13) constructed a multi-epitope antigen fusion (FanCSTa-E2) against *ETEC* and the homologous bovine viral diarrhea virus. They showed that this fusion antigen can potentially be developed as a vaccine for broad protection against bovine diarrhea.

In this study, FanC antigen of Iranian *ETEC* was isolated, it was cloned and subcloned into an expression vector and expressed in *E. coli* strain BL21 (DE3); the purified protein was injected to hens to produce anti-FanC IgY. We immunized the hens before the start of the laying period in order to avoid reduction in egg laying capacity by immunization because the adjuvant use in the immunization can directly decrease this capacity (23). Anti-FanC IgY antibody was produced by immunizing hens with the recombinant FanC protein, which can be used as a tool for the immune diagnosis. The results of this study are in agreement with the results of other studies that indicated immunized hens are an excellent alternative for production of polyclonal antibodies (24, 25). Also, the present results are the first molecular study on cloning and expression of FanC (K99) antigen of Iranian *ETEC* and they agree with results of the other studies that revealed *E. coli* expression system can be appropriate for expression at the high level of FanC antigen of *ETEC* (13, 26). The antigen FanC amount showed that it simulated the immunological response. It was reported the ideal does for simulating immune system ranges from 10 μg to 1 mg (27). In this study, FanC antigen concentration was within this ideal range. The antibody activity started to increase two weeks after intramuscular injection (P-value<0.05). Some studies indicated that anti-*E. coli* IgY activity increased two
weeks post immunization (P-value<0.05) but decreased 3~4 weeks later (21, 25 and 28); this phenomenon appeared in the fourth week in the present study, but it wasn’t significant and started to reach a constant level. The iELISA results indicated that the recombinant FanC protein was recognized by anti-FanC IgY specifically.

**Conclusion**

The results of this study indicate that anti-FanC IgY antibody can be applied in immunotherapy for ETEC infection. Therefore, the anti-FanC IgY could be developed for diagnosis and treatment of ETEC infection. A further experiment with different titers of IgY and its preventive effects on the bacteria may give more practical information for an alternative application of IgY in the future as a supplement to passive immunity in newborn calves, sheep, and pigs against an economically important bacterial disease.

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**Conflict of Interest**

The authors declare no conflict of interest.

**References**

1. Jay CM, Bhashkaran S, Rathore KS, Waghela SD. Enterotoxigenic K99 Escherichia coli attachment to host cell receptors inhibited by recombinant pili protein. Vet Microbiol 2004; 101:153-160.
2. Altmann K, Pyliotis NA, Mukkur TK. A new method for the extraction and purification of K99 pili from Enterotoxigenic Escherichia coli and their characterization. Biochem J 1982; 201: 505-513.
3. Clarke SC. Diarrhoeagenic Escherichia coli an emerging problem? Diagn Microbiol Infect Dis 2001; 41:93-98.
4. Younis EE, Ahmed AM, El-Khodery SA, Osman SA, El-Naker YF. Molecular screening and risk factors of Escherichia coli and salmonella spp. in diarrheic neonatal calves in Egypt. Res Vet Sci 2009; 87:373-379.
5. Lee JH, Isaacson RE. Expression of the gene cluster associated with the Escherichia coli plius adhesion K99. Infect Immun 1995; 63:4143-4149.
6. Roosendaal B, Damosiaux J, Jordi W, de Graaf FK. Transcriptional organization of the DNA region controlling expression of the K99 gene cluster. Mol Genet 1989; 215: 250-256.
7. Kobayashi C, Yokoyama H, Neguyen AA, Baidoo SK. Passive protection effect of egg yolk antibodies against recombinant Cryptosporidium parvum P23 protein. Iran J Parasitol 2009; 4:15-24.
8. Nikbaldt G, Jahitigh M, Assadian F. Generation of egg yolk antibodies in chicken (IgY) against influenza M2 (M2e) protein. International Conference on Chemical, Biological and Medical Sciences (ICCBMS’2012). 2012 August 25-26; Kuala Lumpur (Malaysia): 2012. p. 20-22.
9. Vega C, Bok M, Chacana P, Saif L, Fernandez F, Parreño V. Egg Yolk IgY: Protection against Rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves. Vet Immunol Immunopathol 2011; 142:156-169.
10. Bradford MM. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-254.
11. Polson A, von Wechmar, MB, van Rgenmortel MH. Isolation of viral IgY antibodies from yolks of immunized hens. Immunol Commun 1980; 9:475-493.
12. Klimentzou P, Paravatou-Petsotas M, Zikos C, Beck A, Skopeliti M, Czarnecki J. Development and immunochemical evaluation of antibodies Y for the poorly immunogenic polypeptide prothymosin alpha. Peptides 2006; 27:183-193.
13. Hashish EA, Zhang C, Ruan X, Knudsen DE, Chase CC, Isaacson RE, et al. A multi epitope fusion antigen elicits neutralizing antibodies against Enterotoxigenic Escherichia coli and Homologous Bovine Viral Diarrhea Virus in vivo. Clin Vaccine Immunol 2013; 20:1076–1083.
14. Acres SD, Babiuk LA. Studies on rotoviral antibody in bovine serum and lacteal secretions, using radioimmunoassay. J Am Vet Med Assoc 1978; 173:555-559.
15. Parreño V, Marquardt RR, Jin LZ, Kim JW, Fang L, Frohlich X, Hideaki Y, Peralta RC, Roger D, Sadako S, Yutaka I, Reddy AR, Jin DE, Fang J, Zhang C, Saif L, et al. Milk supplemented with immune colostrum: Protection against rotavirus diarrhea and modulatory effect on the systemic and mucosal antibody responses in calves experimentally challenged with bovine rotavirus. Immunol Immunopathol 2010; 136:12-27.
16. Shahbazi P, Shayan P, Ebrahimzadeh E, Rahbari S. Specific egg yolk antibody against recombinant Porcine Epidemic Diarrhea Virus COE protein. Acta Vet Sc 2014; 42:1181.
17. Borhani K, Mohabati-Mohabat A, Khabiri AR, Behmanesh M, Khoramabadi N. Production of specific IgY Helicobacter pylori recombinant OipA protein and assessment of its inhibitory effects towards attachment of H. pylori to AGS cell line. Clin Exp Vaccine Res 2015; 4:177-183.
18. Hsu Q, Zhang X, Li X, Xu F, Lv Y. Production of Egg Yolk Antibody (IgY) against recombinant Porcine Epidemic Diarrhea Virus COE protein. Acta Vet Sc 2014; 42:1181.
19. Nasiri et al. Specific IgY antibody to recombinant FanC protein
22. Malekan M, Moosakhani F, Pourtaghi H, Bahonar A, Malekan M. Production of immunized eggs against Salmonella enteritidis and Escherichia coli k99. Vet Clin Path (Vet J Tabriz) 2010; 4:759-764.
23. Schade R, Calzado EG, Sarmiento R, Chacana PA, Porankiewicz-Asplund J, Terzolo HR. Chicken egg yolk antibodies (IgY-technology): a review of progress in production and use in research and human and veterinary medicine. Altern Lab Anim 2005; 33: 129-154.
24. Nasiri K, Nassiri MR, Tahmoorespur M, Haghparast A, Zibaee S. Production and characterization of egg yolk antibody (IgY) against recombinant VP8-S2 antigen. Pol J Vet Sci 2016; 19: 271-279.
25. Liou JF, Shiau JW, Tai C, Chen LR. Production of egg yolk immunoglobulin against Escherichia coli from white Leghorn and Lohmann chickens. J Anim Vet Adv 2011; 10:2349-2356.
26. Yuan P, Yanshun C, Zhiqiang S, Xiaoxiang N, Jinliang W, Guangjun G, et al. Co-expressions of fimbrial subunit gene K88 and K99 of Enterotoxigenic Escherichia coli. Chin J Vet Sci 2009; 29:437-440.
27. Schade R, Behn I, Erhard M, Hlinak A, Staak C. Chicken egg yolk antibodies, production and application: IgY-Technology. Germany: Springer; 2000.
28. Yokoyama H, Peralta RC, Diaz R, Sendo S, Ikemori Y, Kodoma Y. Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic Escherichia coli infection in neonatal piglets. Infect Immun 1992; 60: 998-1007.