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Research Article

Exploring the Immunoglobulin Y (IgY) Technology for the Production of Polyclonal Antibodies for Targeted Pathogens of Livestock for Possible Development of Biomolecules in Disease Diagnostics

Kekungu-u Puro[1]*, Rebecca Marbaniangm[2#], Heibor Shilla[1], Sabia Khan[1], Zakir Hussain[1], Umjer Ramshon[1], Arun Prince Milton[1], Samir Das[1] and Sandeep Ghatak[1]

[1]*Division of Animal and Fisheries Sciences, ICAR-RC for NEH Region, Umiam, Meghalaya, India*

[2]*Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Chennai, India*

***Corresponding Author:** Kekungu-u Puro, Division of Animal and Fisheries Sciences, ICAR-RC for NEH Region, Umiam, Meghalaya, India.

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Abstract

Exploring Immunoglobulin Y (IgY) technology for the production of polyclonal antibodies offers exciting possibilities, particularly in the context of livestock health and disease diagnosis. IgY is the primary antibody found in the egg yolk of birds, such as chickens and presents several advantages over traditional mammalian antibodies. We presented here the production of IgY antibodies in eggs of hyperimmunized rainbow rooster birds against heat inactivated Brucella organism (Brucella abortus and Brucella melitensis) which is an economically important pathogens for livestock with zoonotic implications. The IgY protein extracted from the yolk of immunized eggs was found to be in the range from 1.016mg ml^{-1} to 4.493 mg ml^{-1} concentration. The anti-brucella IgY produced can suitably be use to explore and developing an affordable diagnostic kit to detect brucella antigen for screening of brucellosis in livestock.

Keywords: Antibodies; Brucellosis; Diagnosis; Immunoglobulin

Introduction

The IgY (Immunoglobulin Y) technology was introduced to describe a procedure consisting of immunization of laying hens with antigen in order to produce polyclonal antibodies of the Y class (IgY). Though the origins of the IgY technology dates back to the 1893, when Klemperer demonstrated that immunized hens (*Gallus domesticus*) generated antibodies against bacterial toxins in the egg yolk [1]. Recent IgYs developed in poultry and isolated from the egg yolk are being used as specific laboratory tools, especially for detecting biomolecules in biological specimens through various *in vitro* techniques and also as *in vivo* immunotherapeutic agents [2]. The distinct structural features of IgY offer several functional advantages rendering IgY a versatile and invaluable *in vitro* tool in biotechnological research, disease diagnostics and *in vivo* immunotherapy. There is no activation of the mammalian (including human) complement system and reaction with mammalian Fc receptors [3]. It is also more resistant to low pH, high temperatures and proteolytic degradation; thereby making it desirable use to detect and neutralize pathogens without activating the host's native defense system [4]. IgYs can be isolated in large quantities from immunized egg yolk and has been applied to various fields of biotechnology and biomedicine [5]. This biomolecule has been used in detection of various human diseases. In livestock, one of the highly infectious zoonotic disease of animals - brucellosis caused by *Brucella* spp. is of significant public health and socio-economic concerns. In India, brucellosis is endemic and the analysis revealed that brucellosis in livestock is responsible for a median loss of US $ 3.4 billion [6]. The eradication of the disease relies on the proper implementation of eradication programs and continuous surveillance to control the spread of the disease [7]. Hence, rapid, simple, and user-friendly diagnostic systems are required in veterinary hospitals and field laboratories for early diagnosis, investigation, and surveillance of brucellosis. Here, we presented the production of IgY antibodies against whole inactivated Brucella species (*Brucella abortus* and *Brucella melitensis*) in immunized eggs and its extraction with the intention to use the antibodies for detection of brucella antigen in a diagnostic platform.

Materials and Methods

Study location

The *in vitro* culturing of bacteria, processing and extraction of antigen, antibody etc were done in the laboratory of Division of Animal and Fisheries Sciences, ICAR-RC for NEH Region, Umiam while the *in vivo* hyperimmunization experiment on poultry bird was carried out in the Poultry Demonstration Unit of Division of Animal and Fisheries Sciences, ICAR-RC for NEH Region (Coordinates: 25.6768° N, 91.9270° E). The experiment was done during July 2023 to August 2024.

Antigen

The production of bulk quantity of brucella spp. antigen (*Br. abortus* and *Br. melitensis*) was done in BHI broth (Hi-Media, India) at 37 °C for 24 hours in a shaker incubator. The brucella spp. were collected by centrifugation (Allegra 64R centrifuge, Beckman Coulter) as per the standard protocol for pelleting and reconstituted in phosphate buffered saline (pH 7.2). Inactivation of brucella organism was done by heating at 97 °C for 30 min. The inactivation was check by sterility test as described [8]. Quantitation of heat inactivated brucella whole protein was done by NanoDrop 1000 (Thermo Scientific) spectrophotometer to determine the concentration and calculated as mg litre^{-1}.

Laying hens

The experimental poultry birds (Rainbow rooster) at point of laying were use for hyperimmunization as per the method described [9] with inactivated brucella antigen for production of immunized eggs by using Freund's complete adjuvant (Difco, India) mixed with 100 µg of antigen per bird by intramuscular injection at multiple sites for the first dose. The subsequent two booster doses after 7 days gap was done using Freund's incomplete adjuvant (Difco, India) with 100 µg of antigen per bird.

Immunized eggs

The eggs laid by the hyperimmunized birds were collected and kept at 4 °C in the refrigerator for further use.

Isolation of IgY from egg Yolk

The extraction of IgY from egg yolk was done as per the method described by [10] with slight modifications. The egg yolk was separated from the albumin and diluted with 2 volumes of distil water. It is then homogenized at 130 rpm (R-8C, REMI) for 5 min. Then centrifuge at 3400 g (Allegra 64R centrifuge, Beckman Coulter) at 4 °C for 30 min. The supernatant was collected and diluted with 3 volumes of distil water and kept overnight for the precipitation of phospholipids and lipoproteins, then centrifuged at 3400 g at 4 °C for 30 min. The resulting supernatant was collected and concentrated using dialysis membrane (50 KDa cutoff, Sigma) and Poly-Ethylene Glycol (PEG) 6000 (Sigma). The concentrated IgY protein sample was precipitated using 20% Ammonium Sulphate $(NH_4)_2SO_4$ and 15% Sodium Chloride (NaCl) in 1:1 ratio, then centrifuge at 3400 g (Allegra 64R centrifuge, Beckman Coulter) at 4 °C for 30 min. The pellet obtained is reconstituted in 1 ml of distill water and stored at 4 °C for future use.

Determination of concentration and purity of IgY protein

The concentration and purity of the extracted protein samples was checked and quantified using NanoDrop 1000 (Thermo Scientific) spectrophotometer.

Visualisation of IgY protein

The IgY protein isolated was visualized by SDS-PAGE as per the method [10] with slight modifications. The 10% nonreducing SDS-PAGE was carried out using a omni PAGE Mini Vertical Protein Electrophoresis System (Cleaver Scientific Ltd) with BSA protein (Amresco) and Protein Molecular Weight Standards (board range) (Thermo Fisher Scientific). The staining was done with Coomassie blue R-250 for overnight and then destained for 2 hrs. The destained gel was viewed under white light with E-Box (Vilber) gel documentation system.

Results

The Brucella organism grown overnight in BHI broth was collected and the MacFarlane OD was determined (0.33 for *Br. abortus* and 0.43 for *Br. meletensis*) and inactivated by heat. The proper inactivation of *Brucella* spp. was checked by sterility test in blood agar and BHI agar growth medium (Hi-media) at 37 °C for 48 hours in an incubator. No growth in both the media confirms the proper inactivation of the brucella spp. The concentration of the brucella antigen was determined (15.421 mg l^{-1} for *Br. abortus* and 34.204 mg l^{-1} for *Br. meletensis*) and used for hyperimmunization of birds as per the schedule dosage and dose. The immunized eggs produced were collected for the separation of IgY as per the method described. The IgY protein concentration was found to range from 1.016 mg ml^{-1} to 4.493 mg ml^{-1} with an average of 1.357 mg ml^{-1} for *Br. abortus* and 1.633 mg ml^{-1} for *Br. melitensis.* The protein purity as per the ratio of 260/280 ranges from 1.50 to 1.52. The SDS-PAGE separated IgY protein (~180 kDa) was visualized in Coomassie blue R-250 stain using protein standard marker as reference.

Discussion

The results showed that it is possible to generate IgY antibodies from chicken eggs by hyperimmunization with *Brucella* spp. The advantages of IgY Technology includes non-invasive collection from egg yolks, avoiding the need for blood collection in animals, which is less stressful and more humane [11]. Chickens can produce large quantities of IgY, enabling the production of polyclonal antibodies in significant amounts [12]. However, the purity and yield of chicken IgY can vary greatly from method to method but extraction of IgY from egg yolk by a precipitation method using PEG is cost-effective and involves two major steps: removal of the lipids

and then precipitation of the total IgY from the supernatant [13]. Our results showed that time of egg production post immunization of antigen influence the yield. Also the yield differ (data not shown) with two different strain of Brucella antigen (*Br. abortus* and *Br. meletensis*). The selection of chicken breed for IgY production is important for obtaining high antibody yield. It has been reported [14] that Rhode Island Red chicken eggs yielded more than double quantities (8.37 mg ml^{-1}) of yolk IgY compared to Single Comb White Leghorns egg (3.66 mg ml^{-1}) which was higher than our findings may perhaps be attributed to strains of birds [14], extraction methods [15] or egg production time post immunization as observed by us. IgY antibodies are stable and can be stored for extended periods without significant loss of function [4]. We have successfully extracted chicken IgY from egg yolks of hyperimmunized hens which can be used for the development of diagnostic reagent for brucella spp detection and has the potential of expanding to other livestock diseases. High production volumes, low cost of production and high selectivity of IgY has significant advantages to enable development of innovative and strategic products [16]. The stability and ease of use of IgY will make it suitable for on-site diagnostic applications, which is crucial for timely decision-making in livestock disease management.

Conclusion

The use of IgY technology for the production of polyclonal antibodies holds great promise for improving livestock health through development of simple, innovative and reliable diagnostic tools for targeted pathogen control program. Continued research and collaboration among veterinarians in the field and researchers in the laboratory will be the key to realizing the full potential of developing biomolecules for managing livestock health and disease control.

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Conflict of Interests

All the authors declared no potential conflict of interest with the research, authorship and publication of this manuscript.

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