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Review Article: Chicken Immunoglobulin IgY Preparation, and Its Applications in Prevention and / or Control of Some Microbial Affections

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AVIAN eggs are one of the largest sources of nutrients required for the development of embryos (proteins, fat, vitamins, growth factors, minerals and a great number of defense factors to protect embryo and neonates against specific infectious agents that affect their mother or against the received vaccine. The yolk of chicken eggs contains the immunoglobulin IgY which is the main antibody in the blood of birds, amphibians, and lungfish. IgY plays the role of mammalian IgG. IgY antibodies are passed from the mother hen serum to their eggs. The recently adopted technological methods facilitate the production and separation of specific highly purified IgY in hen's egg yolk, to be used as an alternative for antibiotics, and conventional serum-based techniques of antibody generation. Hens as IgY factory are kept under normal conditions of life and management without any stress, and they produce eggs containing the desired antibodies. Hens can be immunized by means of s.c or i. m by injection of 0.5-1.0 ml of antigen, to obtain antibody titers ranging from $1/10^4$ - $1/10^6$ after one or 3 - 4 booster doses. Prepared IgY can be stored active under cool or in dried form for about a year, and can administered to birds orally in feed or water as well as by injection.

Our review as part of our Master's degree in poultry diseases, mainly focuses on the collection of published data on hen immunization, collection of eggs, preparation, separation, purification, and usage of the specific egg yolk IgY in poultry restoratives against different pathogens, maintain healthy respiratory, oral, and digestive systems, and also, denote its value in disease prevention and treatment in the field of poultry industry.

Keywords: IgY, Separation, Identification, Therapeutic use of IgY, Routs of administration, Chicken.

Introduction

Immunoglobulin Y (IgY) or egg yolk immunoglobulin (IgY antibody), is the only one in the yolk that transfers from hen's serum to their egg yolk to transmit passive immunity to chicken embryos and newly hatched chicks [1,2]. After immunizing the laying hens, the specific IgY antibody is transferred to the egg yolk, and thus by using several methods it was possible to separate the IgY while preserving the life of the chicken as a safe antibody factory. The hen lays an average of 280 eggs per year for the first, and the yolk of one egg contains approximately 100-150 mg of IgY per yolk that depends on size of yolk and the age of the hen as well as the time after vaccination.

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Therefore, it is expected to obtain more than 40 g of IgY/ year from a hen [3].

It is noted that specific IgY antibodies begin to accumulate in the yolk of chicken eggs by the end of the third week of immunization, when the product can be safely collected [4]. Hakalehto [4] presented specialized antibody solutions prepared from chicken egg yolk antibody (IgY) in both prevention and treatment of many infections due to viruses and bacteria in animals and humans. Modern technologies have now facilitated the production of antibodies IgY from the yolk of chickens immunized with certain antigen, and these methods and techniques have developed to be an alternative method to the old traditional methods of producing serums and antibodies. The modern methods of the IgY antibody production from the eggs are less stressful for the hen with the keeping of laying hens in their natural conditions for laying the eggs, and preserved of hen's life [5].

Application of IgY in prevention of SARS-CoV-2, influenza, and Rota viruses, as well as eradication of *P. aeruginosa*, tooth decay, gut bacteria, toxins and pathogens has been recorded. Oral IgY has been shown to be successful in the treatment of several gastrointestinal human and bovine viral (e. g Rota and Corona viruses) as well as bacterial infections (e.g *E. coli*, *E. tarda,* Salmonella spp., Staphylococcus, Pseudomonas and *Y. ruckeri* [3]. Also, IgY antibodies have been used successfully against many humans and animals viral and bacterial respiratory infections [6, 7].

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Definition and description of IgY

Immunoglobulin Y (IgY) or chicken egg yolk immunoglobulin (IgY antibody), is the only one in the yolk and the immunoglobulin transfers from hen's serum to the yolk to provide passive immunity to embryos and hatchlings [1, 2]. IgY begins to be transferred to the yolk about 21 days after the chickens have been vaccinated and from that time it can be collected.

Comparing chicken IgY and mammalian IgG antibodies

When comparing chicken egg yolk IgY antibodies with mammalian IgG, Chicken IgY has a higher ability to bind to specific antigens, is easy to extract at lower costs, and has, therefore, a high and remarkable ability to neutralize pathogens in respiratory system [6].

Research has concluded that laying hens are an important source of specific and high-quality IgY antibodies, as readily available from the egg yolk. The usage chicken IgY has many special characters as it is non-toxic, environmentally friendly, and maximum lowered number of animals required for antibody production [8]. It also notes that IgY does not attached with rheumatoid factor or interact with mammalian complement factor. All these factors and differences make the application of IgY more broader [1].

Preparation of pure specific chicken IgY
Immunization of Hens

In an attempt to hyper-immunize hens for IgY preparation against both Avian influenza and Newcastle, by using 150 vaccinated laying hens and 30 non-vaccinated control, at 35 weeks of age. The inactivated AI + ND bivalve vaccine was injected into 3 different sites with a dose of 1 ml three consecutive times 2 weeks in between. Blood and eggs samples were collected from vaccinated birds at zero time from the given vaccine, then weekly from vaccinated and control birds. The blood was left at 24-25 °C for clotting, and the sera were separated. Eggs were chosen, collected, cleaned and stored at 4 °C to be used for IgY preparation. Serum antibody titers were set to confirm increased antibody levels after booster

TABLE 1. Differences between immunological characters of Avian IgY, and mammalian IgG.

Interaction	IgY	IgG
Interference with mammalian IgG	No	Yes
Interference with rheumatoid factor	No	Yes
Interference with human anti-mouse IgG antibody	No	Yes
Activation of mammalian complement	No	Yes
Protein A\G binding	No	Yes
Mammalian Fc receptor binding	No	Yes
Absorption from intestine	Yes	No

doses [9].

Methods of separation

Separation of yolk lipids is the main problem in preparation of IgY. There are several methods are adapted to separate immunoglobulin, including water dilution (WD), polyethylene glycol (PEG), dextran sulfate (DS), dextran blue, chloroform, and xanthan gum (Xan) [10, 11]. There are also several methods for purifying immunogens such as Lowry assays, SDS-polyacrylamide gel and MIC assays. [12, 13]. Some of these methods will be mentioned below.

- *Precipitation of Chicken IgY from Egg Yolk by Polyethylene Glycol (PEG)*

Polson et al. [14] adopt method for precipitation of chicken IgY. Wear latex gloves at all steps, start by cracking the eggshell after cleansing. Transfer the yolks to a spoonful of yolks and make sure to get rid of the egg whites. Wrap the yolks in filter paper to remove the remains of the whites. Cut the yolk membrane with a scalpel or tool. Pour the yolk into a 50 ml tube, measuring its volume. Mix yolk with PBS in a ratio of 1:2 after adding 3.5% PEG 6000 of the total volume and rotating in cooling (4°C) centrifuge for 20 min (10,000 rpm according to 13,000 x g, Heraeus Multifuge 3SR+, fixed angle rotor), followed by rolling for10 minutes on mixer. This extraction procedure separates the suspension in 2 phases. The first phaseis yolk solids and fatty component, while the 2nd is the watery phase that containing other proteins and IgY [14].

The supernatant watery phase must passed through a filter and descant to clean sterile tube with 8.5 % PEG 6000/gram (calculated the new volume), vortexes and rolled on a rolling mixer. Carefully dissolve the pellet in 1 ml of PBS by a glass rod and a vortexer. Add PBS up to 10 mL. This solution was mixed with 12% PEG 6000 (w/v, 1.2 g) and processed as in step 3 (vortex, tumble mixer). Repeat step 6 to gently dissolve the pellet in 800 µL of PBS (glass rod and vortex). Wait for till completely disappearance of air bubbles, and transfer (pipettor) the extract into the dialysis capsule. Rinse the tube with 400 µL of PBS and transfer the volume to the dialyzer. Dialyzed the extract overnight against 0.1% saline (1600 mL) and gently stirred with magnetic stirrer. At morning, PBS must replace the saline and dialyzed for more 3 hours. The IgY extract was then pipetted from the dialysis capsule and transferred to a 2 ml tube. The protein content (mg/mL) was must be diluted 1:50 with PBS and photometrically measured at 280 nm. Calculate the concentration according to the Lambert-Beer law. Where, extinction coefficient of IgY was 1.33, indicating the preparation (purity recovery at 80 % about). It is recommended to store sample aliquots at temperatures not exceeding -20 °C [14].

- *Separate IgY from egg yolk without using organic solvents*

Egg yolks were separated from egg whites, double dilute its volumes with cold (4°C) DW and homogenized using a hand mixer (Kitchen Aid) at high speed (setting 9) for 1 min. After centrifugation at 3,400 xg for 30 min at 4°C, the supernatant was passed to isolate IgY and the pellet was used to isolate phosphorproteins.

The pooled supernatants were diluted with 3 volumes of 4 °C DW, placed in the refrigerator overnight to pellet both lipoproteins and phospholipids, followed by30 min centrifuged at 3,400 × g at 4 °C. The supernatant was concentrated via ultrafiltration (membrane filter cut-off: 50 kD, GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Precipitate IgY from the concentrated solution using a combination of 20% saturated ammonium sulfate (final concentration) and 15% NaCl (w/v, final concentration). Precipitates were collected after centrifugation at 3,400 × g for 30 min at 4 °C, dissolved with 9 its volumes with DW, and re-precipitated with combination of (NH4) $2SO_4$ + Na Cl to increase purity. The precipitate was dissolved with 9 volumes of DW, desalted by ultrafiltration, and freeze-dried (Free Zone Freeze Dryer, Labconco Corp., Kansas City, MO) [15] .

- *Extraction of egg yolk IgY-antibodies by ammonium sulphate-caprylic acid*

Egg yolk must be separated from the egg white. DissolveEgg yolk in 1:4 with PBS pH 7.5, then readjust pH to 4.6, and add 6% Caprylic acid (v/v)to precipitate the non-immunoglobulin proteins. Discard the obtained precipitates just following 30 m centrifugation at 14000 ×g collect the supernatants and adjust the pH to 7.5 with 1 M Tris-buffer. IgY-antibodies Extractions must be done according to Almeida et al. [16]. Biuret method must be used to measure the protein concentration followed by filtration of the prepared IgY by 0.45 µm filter and stored at 4°C

- *Production and purification of egg yolk IgY by ammonium and sodium sulphates*

Water dilution method was used to obtain purified IgY [17]**.** Egg yolk was separated from

the white, albumin was totally removed by washing with distilled water and followed by rolling of yolk on paper towel to remove much adhering albumin. The yolk membrane was opened and pours the yolk content in a graduated cylinder. The separated yolk was 1/10 diluted by its volume with distilled water (acidified with 100 mM HCI, and adjust the mixture pH to 5.0. The diluted yolk must be hold for 6 hrs followed by cold filtration through filter paper (Whatman no.1). The obtained IgY filtrate must be purified by successive precipitations with 33% ammonium sulphate, 18 and 14% sodium sulphate. The purified IgY.(> 96% purity) must diluted with PBS and freeze dried.

Identification and quantity measures:
-SDS-PAGE for IgY:

IgY was subjected to a non-reducing SDS-PAGE using a Mini-Protein Tetra cell from Bio-Rad. Coomassie Brilliant Blue R-250 staining and a 10% SDS-PAGE electrophoresis were applied. Using the Gel Doc (Bio-Rad), the protein bands in the gel were examined after destination [18]. IgY and phosvitin purity estimates were made using SDS-PAGE, and the purity of IgY was determined by converting density of protein bands in the gel using Image program (National Institutes of Health, Bethesda, MD).

-ELISA Assay of Separated Proteins:

An ELISA assay was performed to check the purity of the purified intact protein after lyophilization using ELISA. A standard curve was constructed using serially diluted IgY and Phosvitin standards (IgY from MP Biomedicals and Phosvitin from Sigma-Aldrich). Each standard and purified protein was dissolved in 50 mM carbonate buffer (pH 9.6) and diluted to a final protein concentration of 10 µg/ml. Using the methods of Ko and Ahn [19] and Abeyrathne et al.,[20], 100 µl of diluted standards and samples were applied to the microplate and incubated overnight at 4°C.3-Western Blot of IgY and Phosvitin:

- Western blot of IgY and phosvitin

IgY and phosvitin were confirmed to have been isolated using a Western blot[21]. Proteins were poured onto a nitrocellulose membrane (Bio-Rad) after the SDS-PAGE was performed according to methods of Naas [23] and Kim [23] at 90 V for 2 hrs at of 4°C. A 5% solution of skim milk powder, dissolved in PBS with Tween-20 was used to prevent the transferred membrane (PBST). Anti-chicken IgY (HRP; US Biological, after 1:15,000 dilution was applied to the membrane to detect IgY, and it was then left overnight at 4°C with shaking. The membrane was subjected to Amersham ECL Prime (GE Healthcare) for 5 minutes after being washed three times with the PBST solution at 10-minute intervals, and it was then analyzed with a Chemidoc. (Bio-Rad).

Mode of IgY action
-Agglutination:

IgYs cause agglutination of pathogens including different variety of virus, bacterial, and fungal, with their immobilization and facilitates their removal from the intestine[24]**.**

-Adherence-blockade

The main mechanism of IgY action against organisms in vitro has been confirmed to be the inhibition of adhesion [25.26]. In vivo experiments revealed that IgY prevented E. coli K88 from adhering to piglets' intestinal mucus. Additionally, it was found that IgY might bind to elements that are exposed on the surface of gram-negative bacteria, such as flagella, lipopolysaccharides, fimbriae (or pili), and outer membrane proteins [27]. The function of these bacteria-related components those may be involved in growth was impeded or prevented by the binding of the IgYs. Additionally, it's conceivable that specific IgY binding to bacteria could change the cell's communication mechanisms and result in a reduction in toxin synthesis and release [28].

-Opsonization followed by phagocytosis

Studies have shown that IgY enhances phagocytic activity against invading pathogens. IgY improved phagocytosis of *Staphylococcus aureus* (*S. aureus)* and *E. coli* by macrophages and polymorphonuclear neutrophil leukocytes. Also, the specific IgY binding on the surface of *S. typhimurium* [26] and *E. coli* O111 lead to structural changes that can be detected using the electron cloud and/or electric field on the surface of bacteria [26]. Addition of IgY to a bacterial suspension causes bacterial stabilization and agglomeration and in minutes of obliteration [29].

-Neutralization

S.aureus invasion to mammary epithelia was inhibited with IgY by neutralizing the bacterial toxins. Also, IgY can inhibit the spread of virus particles from cells to other cells, this action prevents colonization of infective viral [24].

Advantages of passive immunization with IgY

Advantages of egg yolk IgY antibodies are numerous [30] including: 1.IgY are produced

naturally away from the effect of different environmental contamination 2.They target multiple antigens. 3. They are directed to target specific antigen with no evidence for induction of specific resistance. 5. IgY does not affect the natural microbial population of the host. 5. IgY antibodies do not accumulate or deposited in meat, therefore its use do not need special regulations in countries those, forbidden the usage of antibiotics in treatment of poultry and livestock [31].

Additional advantages of usage of IgY antibodies for passive immunotherapy are they have both local, and rapid activity on pathogen. IgY is not host specific therefore they can be given to variety of individuals at regardless to age, immune and not affect pregnancy in women [29]. The IgY antibodies are nontoxic and can be stored in dry powder form for months at low moisture without refrigeration as compared with vaccines [24]**.** Moreover, IgY has been found to have a more antigen-targeting binding capacity than that of mammalian IgG [32]. It has also been found that it is easy, successful and requires a small amount of antigens to produce IgY antibodies against conserved mammalian proteins from mammalian IgG production [30,33]. IgY can be stored in eggs for at least 1 year at 4 °C [34]. From one chicken a large quantities of IgY antibodies (about 22 g/ year) can be produced with high specificity**,** which is more than the annual production from 4 rabbits as well as production IgY in large quantities with much lower costs [8, 26, 31,35].

Application of IgY Antibodies in Detection and Immunoassay

Mammalian IgG produce more cross-reactivity than the egg yolk polyclonal IgY. IgY does not bind to the mammalian Fc receptor or cross-react with many proteins or activate complement factors. IgYassay reduce interference by these mammalian factors [3**].**IgY antibodies are polyclonal in nature.Thisnature limited the development in immunoassays applicationsre quiredpurified homogenous IgY antibody preparation.

It was recorded that hybridoma techniques still not correctly established for the of generation avian IgY, while phage display was more efficient in generate recombinant IgY. By phage display, it is possible to use and applythe most useful properties of both IgY and monoclonal antibodies. [36-38].

Usage of chicken egg yolk antibody (IgY)

The use of egg yolk antibody (IgY) solution for the prevention and treatment of certain viral and bacterial infections is a rapid, reliable, safe and tested method for molecular protection and protection against emerging pathogens and epidemics [4, 27, 39].

a. Control of viral infections in poultry

1. in chickens

- Infectious bursal disease (IBD)

Abd El-Ghany [40] compared the efficacy of IgY and vaccines in preventing IBD in chickens and reported that vaccines and IgY were relatively equally effective but their combination was superior in preventing IBDV infection in broiler chickens. Eggs collected from laying hens were used to isolate IgY antibodies from egg yolks by ammonium sulfate-octanoic acid extraction. The mean log10 antibody titers of serum samples increased significantly 2 weeks after immunization and reached a maximum after 6-8 weeks. Yolk IgY antibody levels increased 4 weeks after immunization and peaked at 8-10 weeks after immunization.

Evaluation of the protective value of IBDV-specific IgY antibodies showed a 15% and 10% reduction in morbidity and mortality in challenged chickens compared to 90% and 40% reductions in non-vaccinated challenged chickens. %. In flocks actively immunized with live and inactivated IBDV vaccines, morbidity and mortality were reduced to 10% and 5%, respectively. However, when IBDV-specific IgY antibodies were administered concomitantly with the IBDV vaccine, morbidity and mortality dropped to zero [41]**.** Yusuf et al. [42] reported that IBDV outbreak was controlled by oral administration of IgY to chickens shortly after detection in a poultry farm. IBDV hyperimmune egg yolks were administered via drinking water to susceptible chickens exposed to IBD. The exposed treatment birds had a 66.6% reduction in mortality compared to untreated control. The treated birds were shifted to show less severe symptoms than the nontreated control.

- Avian influenza (AI):

Wallach et al. [43] and Nguyen et al. [44] generated IgY antibodies against AI H1N1, H3N2, and H5N1 viruses and found that by using in vitro mouse assays to test IgY for HI tests and serum neutralization tests The ability of IgY to be both homologous and heterologous in some suppressed cases of clades and virus strains. Using an in vivo mouse model system, we

found that intranasal administration of anti-H5N1 IgY 1 hour before infection protected 100% of mice from lethal H5N1 challenge. Of particular interest is the in vitro and in vivo cross-protection of IgY against H5N1 against A/Puerto Rico/8/34 (H1N1). These results are clearly indicated that anti-influenza virus IgY can be used to prevent AI virus infection. The presence of significant titers of purified IgY against AI V subtypes H5N1 and H9N2 in eggs, and the possible role of egg yolk IgY in neutralizing these viruses [43,45].

- *Detection and Treatment of Reovirus*

Avian reoviruses are associated with many clinical forms, the most frequent are malabsorption syndrome, Arthritis, immunosuppression, Pericarditis, and Myocarditis Osteoporosis in poultry [46]**.** Administration of IgY specific to Reovirus in infected birds advertised a high sensitivity to the virus, facilitate detection of the virus in contaminated tissue, and neutralized the virus in BHK-21 cells. Specific Reovirus IgY was attached only to homologous virus strains [36, 47]**.** Reovirus is an important inducer of growing problem in aquaculture with high mortality rates in crabs. Usage of IgY anti-swimming Reovirus was highly effective in detection of virus in carb samples, the result indicated benefits in reovirus-associated disease outbreaks in aquaculture [37]**.**

- *Newcastle disease (ND) virus*

ND is caused by the virulent virus strains of avian paramyxovirus serotype 1(PMV-1) with severe losses in poultry industry [48]. Gadde et al., [8] recorded the prophylactic and IgY had a therapeutic effects of in ND virus infected chickens. The i. m injection of diluted egg yolk was apple to protect challenged birds for 12 days, while its injection 72 hs post infection prevented the appearance of ND signs. Box et al. [49] reported that a significant rate of protection was obtained from the s.c injection of yolk having high titers of ND virus antibodies. Hamal et al [50] found that hyper-immunized layer chickens with live attenuated ND vaccine showed high yolk antibody levels, these levels were result in more higher yolk antibodies. The higher protection rate was seen in yolk treated 2 week old chicks were protected after 1 week. Nguyen et.al [44] noticed that repeated egg yolk treatment increased the antibody titer. Yegani and Korver [51] recorded that egg yolk containing high ND antibodies titers was apple to protect 80% of birds against NDV. For immunization of birds, the i.m rout was resulted in higher antibody titers [51, 52]. Acidity of and proteases of proventiculus and gizzard deteriorate egg yolk in oral administrated, however only a fraction of the given dose had immunological activity against GIT infection. IgY administration by i.m had a great prophylactic opportunities for increase and maintain the protective levels against ND virus [53,54].

2: in ducks

Cova [55] stated that egg sare considered mini factory Igy antibody production, the specific active IgY are transmitted vertically from bird serum to egg yolk from which pure 60-100 mg can be extracted from an egg yolk of a gene vector DNA-immunized duck [55, 56] . As immune-diagnostic the duck IgY has a special value, because of they do not have cross-react with mammalian IgG and complement [55, 57]. IgY is able to resistant gastric barrier, IgY specific to *H. pylori*are of value for passive immunotherapy in face of GIT infections [55,58,59].

- *Duck virus hepatitis (DVH)*

For control of DVH in recent infected duckling the injection of convalescent serum or egg yolk from hyper-immune breeder ducks, or yolk from SPF collected from DVH supper-immunized specific-pathogen-free (SPF) chickens [60]**.** Subcutaneous injection of ducks at the onset of signs with prepared DHV type-1immune serum or egg IgY of immunized chickens was highly effective in treatment of infected duck flock [61,62].Vaccinated ducklings with Modified-live DHAV-1 via s.c or by foot web stab developed active immunity within3-4 days after vaccination**[**63-65]**.**

- *Cure of bacterial infections in poultry*
- *Escherichia coli (E. coli)*

E. coli infections are considered one of major problems facing poultry industry due to , high cost of prevention and economic losses [66,67]. The efficacy of passive transferred IgY in prevention of *E. coli* respiratory infections in broiler chickens was reported by Kariyawasam et al. [68]**.** Broiler chickens i.m injection with purified IgY from hens immunized with different *E. coli* antigens were protected against the experimental infection with the homologous *E. coli* strain [69]. Chicks that were orally supplemented with specific IgY prior to *E. coli* challenge showed no symptoms or lesions, and colonization of the chick gut with *E. coli* was reduced [70]. Diets supplemented with at least 0.2% IgY egg yolk powder for 3 weeks was result in reduction of ileum *E. coli* count and improved broiler chickens immunity

when infected with E. coli O78:K80 [70,71]. Concentrated IgY targeting *E. coli* and *S. enterica* was demonstrated to be effective in reducing bacterial growth rates in vitro [72, 73]**.**

- *Salmonella*

Salmonella genus is containing Gram-negative enterobacterium with several serotypes causing diseases in human and a broad range of animals. Both *S. pullorum* and *S. gallinarum* are the important serotypes, those induce severe septicemic affection in chickens and turkeys [74-75.76]. Igy have been used to reduce both *S. pullorum* and *S. gallinarum* affections in and also, reduce their ability for colonization cecal and reduce the horizontal transmission of pullorum disease [8]. IgY from hyperimmune hens was given as oral supplementation protected birds from challenge with *S. pullorum* [77]. Lee et al. [25] tested in vitro effects of IgY specific antibodies against chicken SE or ST and found that growth of both Salmonella numbers were reduced. The administration of in capsulated salmonella IgY specific have power to reduced liver and caecal the colonization of in chicken, the author concluded that capsulation is necessary to protect IgY from the gastrointestinal degradation [73].

- *Campylobacter*

C. jejuni is a normally inhabits the large intestine of chickens [78]. Alternatives to antibiotics IgY antibodies was used to reduce campylobacter cecal colonization in poultry to reduce chicken meat contamination by Taha-Abdelaziz et al [7]**.** In another trial carried out by Thibodeau et al.[80] stated that egg IgY solution was given orally just before *C. jejuni* infection, reduced both chicken morbidity and mortality as compared with the non-medicated group. Hermans et al. [81] recorded a reduction in *C. jejuni* counts in broiler chicks cecum after feeding diets containing dried IgY in rate of 5% (w/w) and noticed that therapeutic efficacy of the IgY was time limited due to the return of *C. jejuni* to normal with stop of the treatment.

- *Clostridium perfringens (C. perfringens)*

C. perfringens is the cause of Necrotic enteritis (NE) in chickens especially broiler type, and imposing high economic losses among poultry industry [82,83]**.** Wilkie et al. [84]notice that in feeding broiler chicken on diet with anti-*C.perfringens* egg antibodies the intestinal count of *C. Perfringens* was not affected. While, the record of Tamilzarasan et al. [85] reported a reduction in both bird's mortality and morbidity,when the birds were given IgY before *C. perfringens* challenge..

2: in ducks

Egg IgY was proved to protect ducks against the following serious bacterial infections:

-*Gallibacterium anatis*

This bacterium is Gram-negative of family Pasteurellaceae and normally inhibited poultry respiratory and reproductive tracts. An Anti-GtxA-N IgY antibody injected into chickens was capable to protect them against infection with *G. anatis*, where, both postmortem liverand duodenum lesion scores were markedly lowered [86]**.**

- *Riemerellaan atipestifer (RA)*

In contrast with antibiotics and vaccines, IgY can be simply and economically obtained with average of over 60−100 mg/ a single egg **[**55]. In a study, laying hens were vaccinated injected 3 times with inactivated RA vaccine isolated anti-RA IgY was obtained and its efficacy for both immunity and clinical treatment were estimated in Muscovy ducks [87].

- *Cure of parasite infection*

Control of Eimeria infection

Unfortunately, numerous Eimeria strains accept resistances to anticoccidials [88]**,** therefore, both attenuated vaccines [89] and IgY antibodies against Eimeria was explored as preventive and therapeutic tools [90]. IgY was protected for chicks till the 3rd weeks of age [43]. In administration of a commercially egg yolk specific IgY powder against 3 different Eimeria species to chicken diet the intestinal lesionswere reduced **[91].**In other study, it was recorded that the application of IgY aginst 5 species of Eimeria for treatment of chicks against *E. tenella* experimental infection, the following parameters were recorded where mortality, caecal lesion score and oocyst shedding were reduced with marked increased body weight gain [92].

Methods of IgY administration

a. IgY in Oral

Anti-Streptococcus mutant IgY was highly effective in the local protection against both dental caries and dental plaque, and a good results in its application as a passive protection tool in both control and prevent dental caries in man [93-94-95].

b. IgY in Care Respiratory

Carlander et al [96] and Thomsen et al. [95]

declared the prophylactic advantages of IgY antibodies in face of *P. aeruginosa* infection, while, in vitro trial 25 µg/ml IgY was actively neutralize against Zika virus [97].

c. IgY in Care against different enteric viral and bacterial infections

IgY was preventing some viral infections as bovine rotavirus induced diarrhea in murine modelandprotectingnewborn calves from BRV diarrhea under field condition [98]. IgY protect neonatal calves from Rota virus diarrhea under field conditions [24], Canine Parvo-virus [7], Rabies Virus [99] and SARS-Cov-2 [4]**.**Anti-Ebola virus IgY collected from eggs of hens immunized with a recombinant Ebola virus glycoproteins was effective in protection of new born Balb/c mice infected with the lethal dose of virus [100]**.** It was found that the prepared highly specific IgY anti-nonstructural Dengue virus P1 and virus serotype 2 was neutralized the virus effectively in immunoassays [97,101,102]. Also, IgY was found to be effective against some bacterial infections e,g neonatal calves colibacillosis was prevented by K99-pilated *E. coli* [32] and *E. tarda* infection in Japanese eels [94], and Anti-Helicobacter pylori in humans [58,103].

Conclusions

Chicken egg yolk antibodies (IgY) are compact folded spherical polypeptide chains proteins. Efficiency of the pecific IgY in reducing pathogens in humans, animals and birds was recorded. It's easy to mass-produce chicken IgY against large number of biological agents including toxins and pathogens (Viral and Bacterial).

The prepared and IgY can be used in immune-diagnostics, identification of disease markers, immunotherapy and treatment of specific infections. Opens up the technology to produce and extract massive quantities of Ab IgY for use in human and veterinary medicine for therapeutic/ preventive purposes. Their universal application in both research and medicine is still expected to play an increasing role in immunotherapy in the near future.

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Authors' contributions

Fatma Mohamed Radwan and Ahmed Ali El-Shemycollected data, wrote and revised the original draft. Mostafa Ahmed Bastami and Mohamed M. Amer supervised the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

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Declarations

All data included in this review are collected from published article

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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تحضير الجلوبولين المناعي IgY للدجاج وتطبيقاته في الوقاية و / أو السيطرة على بعض مسببات الأمراض

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يعتبر بيض الطيور أكبر مصدر للمغذيات الرئيسية التي تتكون من أنواع مختلفة من البروتينات والدهون والفيتامينات وعوامل النمو والمعادن اللازمة لنمو الجنين وأيضًا عدد كبير من العوامل الدفاعية للحماية من العديد من الالتهابات الفيروسية والبكتيرية. كما أنه يتكون من مواد بيولوجية مختلفة تشكل الوحدة الأساسية للحياة. يحتوي صفار بيض الدجاج على البروين المناعي (IgY Y) وهو الجسم المضاد الرئيسي الموجود في الطيور وسمك الرئة والبرمائيات وسمك الرئة ، ويلعب دورًا مشابهًا مثل الأجسام المضادة IgG للثدييات. يتم نقل الأجسام المضادة IgY من المصل إلى صفار البيض وتسمى IgY بسبب البروتين المناعي لصفار البيض. تسمح التقنيات الحديثة بإنتاج الأجسام المضادة في صفار بيض الدجاج ، لتكون طريقة بديلة لتوليد الأجسام المضادة. نظرًا لأن الدجاج يتم الاحتفاظ به في جميع ظروفه الطبيعية تقريبًا ، يتم عزل الأجسام المضادة من البيض الذي تم جمعه. يمكن تحصين الدجاج عن طريق الحقن في العضل ، جرعه الحقن ١,٠-٠,٥ مل. اعتمادًا على مناعة المستضد ، عادة يمكن تحقيق مسوي عالي من الأجسام المضادة (حتى ١: ١٠٠٠٠٠ : ١: ١٠٠٠٠٠٠) بعد تحصين واحد أو ٣-٤ تحصينات معززة ، تضع الدجاجة بيضها بشكل مستمر لمدة ٧٢ أسبوعًا ، وبعد ذلك تقل القدرة على وضع البيض. في الوقت الحاضر ، تم تطوير تحضير الأجسام المضادة (IgY) في صفار البيض بواسطة الدجاج المحصن ليكون بديلاً للتقنيات التقليدية القائمة على المصل لتوليد الأجسام المضادة. يمكن تخزين IgY المحضر نشطًا في مكان بارد أو جاف لمدة عام تقريبًا ، ويمكن إعطاؤه للطيور عن طريق الفم في العلف أو الماء وكذلك عن طريق الحقن.

تركز هذه المقاله بشكل أساسي على تطبيقات الأجسام المضادة لصفار بيض الدجاج (IgY) الخاصة بمستضد معين في العلاجات البشرية والحيوانية للحماية من العدوى البكتيرية والفيروسية المختلفة وللحفاظ على رعاية جيدة للجهاز التنفسي والجهاز الهضمي مما يبرز هذه القيمة. أهميتها لصحة الإنسان والحيوان والوقاية من الأمراض والسيطره عليها وعلاجها بشكل فعال.

الكلمات الداله: IgY ، الفصل ، التحديد ، الاستخدام العلاجي لـ IgY ، الدجاج.