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Review

Egg yolk antibodies (IgY) and their applications in human and veterinary health: A review

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ABSTRACT

Egg yolk constitutes a relevant alternative source of antibodies. It presents some advantages over mammalian serum immunoglobulins regarding productivity, animal welfare and specificity. The main immunoglobulin present in avian blood (IgY) is transmitted to their offspring and accumulates in egg yolks, which enables the non-invasive harvesting of high amounts of antibodies. Moreover, due to structural differences and phylogenetic distance, IgY is more suitable for diagnostic purposes than mammalian antibodies, since it does not react with certain components of the human immune system and displays greater avidity for mammalian conserved proteins. IgY has been extensively used in health researches, as both therapeutic and diagnostic tool. This article aims to review its applications in both human and veterinary health.

1. Introduction

Antibodies are protein molecules produced in response to an antigen. Due to their ability to bind to specific targets, they are widely used in research, diagnosis and therapy. Most of the currently available antibodies are produced in mammals, especially in small rodents [1]. However, the production of antibodies in mammals can be challenging due to the fact that some antigens elicit weak immune responses or are even completely non-immunogenic. Moreover, the production of antibodies in mammals involves procedures that cause pain and distress to animals; such as immunization, blood sample collection and sacrifice [2]. The search for more efficient and economical techniques, as well as for the reduction and refinement of the use of animals, has led to growing interest for egg yolk antibodies (IgY). Obtaining antibodies from egg yolk is a non-invasive method that eliminates the need for blood collection.

Hens produce a greater amount of antibodies compared to other animals, like rodents for example, considerably reducing the number of animals required for the production of antibodies [2,3]. This method presents several economical advantages over the use of other animals. For example, the cost of maintaining a hen is less expensive than maintaining animals like mice and rabbits; the amount of antibodies produced by chickens also corresponds to that of larger animals, such as goats and sheep. For instance, in one year, a hen lays about 300 eggs and produces an average of 18, 25 g of IgY [4]. Moreover, the specific IgY produced in chickens are about 1–10% of the total amount of antibodies [1]. Nonetheless, the amount of antibodies produced is correlated to the quantity of antigen applied to the hen, its immunogenicity and molecular weight [5,6]. Being a productive technique, as well as more refined from the point of view of animal welfare, IgY technology has been used for several purposes in human and veterinary health, such as in immunodiagnostics [7], immunotherapy [8], neutralization of toxins from venomous animals [9] and bacteria [10], and as functional food [11].

Considering the fast development of IgY technology, this work aims to review its applications in human and animal health, in addition to increasing the potential use of these antibodies among researchers and consequently promoting the reduced use of non-invasive procedures on animals.

2. Structural and biochemical properties of IgY

The specific protective effect of egg yolk extracts from immunized hens, attributed to the transfer of serum chicken antibodies to eggs, was first described in 1893 [12]. However, this knowledge remained without applications until it attracted the interest of the scientific community due to the search for animal welfare, which was driven by the works of Russel & Burch and the publication of the “Principles of Humane Experimental Technique” in 1959. The use of IgY increased in the 80s, possibly due to the development of commercially available

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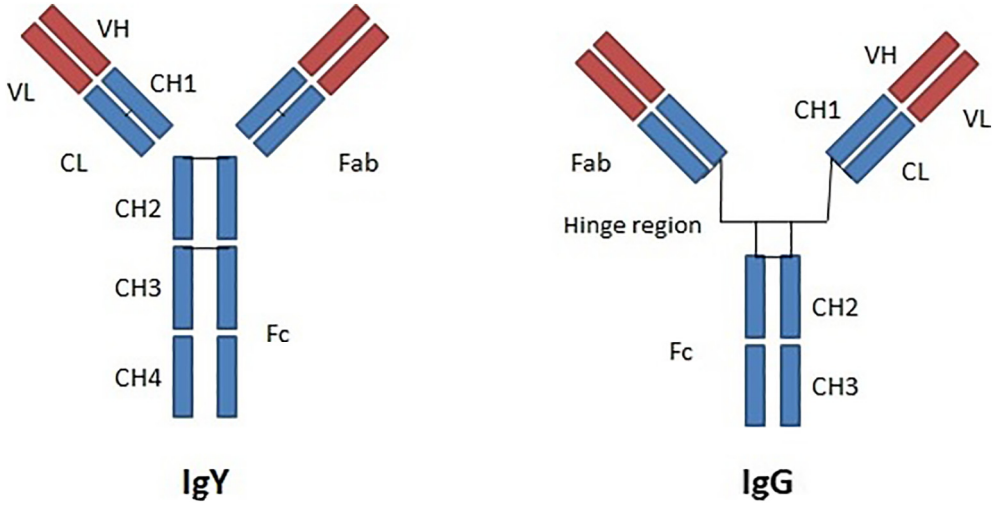

Fig. 1. Structure of IgY and IgG. V = variable domain of the light chain (VL) and the heavy chain (VH); C = constant domain of the light chain (CL) and the heavy chain (CH).

secondary reagents, such as IgY purification kits and anti-IgY specific antibodies conjugated to alkaline phosphatase, fluorescein isothiocyanate and peroxidase markers [13]. In 1996, the European Center for the Validation of Alternative Methods (ECVAM) workshop recommended the use of IgY rather than mammalian IgG, with the purpose of minimizing the pain generated by invasive collection of serum antibodies [3].

IgY is present in birds, reptiles, amphibians and lungfish and is the evolutionary precursor of IgG and IgE, present only in mammals [14]. Over time, IgY was called IgG due to the supposed similarity between the two. However, Leslie & Clem emphasized the distinct differences between IgY and IgG, such as antigenic differences and the major size of IgY heavy chain, suggesting the use of this term instead of IgG [15].

The IgY molecule has a structure similar to that of IgG, with two heavy chains (H), each one with a molecular weight of 67 to 70 kDa, and two light chains (L), with 25 kDa. The light chains have one constant region (CL) and one variable region (VL), similar to IgG. The major difference between IgY and IgG is found at the heavy chains. IgG has three constant regions at the heavy chains (CH1-CH3), while IgY has four constant regions (CH1-CH4) (Fig. 1). A further constant domain, with the corresponding carbohydrate chains, gives IgY a higher molecular weight (180 kDa) in comparison to IgG (150 kDa) [1].

IgY is less flexible than IgG due to the absence of the hinge region between CH1 and CH2 [1], similar, in this aspect, to IgE [16,17]. The presence of glycine and proline residues at CH1-CH2 and CH2-CH3 regions also limits IgY flexibility [1]. One advantage of this higher chain stiffness is that it may be associated with increased IgY resistance to proteolytic degradation and fragmentation [17]. Also, IgY is more hydrophobic than IgG and has an isoelectric point between 5.7 and 7.6. The half-life of purified IgY is of months, retaining activity for up to six months at room temperature and one month at 37 °C. Moreover, affinity-purified and 21 biotinylated IgY retained high activity after five years of storage at 4 °C [1]. The structural and phylogenetic differences between IgY and IgG are manifested in different molecular and biochemical interactions. The Fc portion of IgY is unable to activate the human complement [18] and to bind to rheumatoid factor [19] and to protein G [20], it also does not react with human anti-mouse antibodies (HAMA) [21] nor with the erythrocyte agglutinogens A and B [22]. Due to phylogenetic distance, chickens produce a stronger immune response against conserved mammalian proteins [23]. Although IgY contains the variable (V), junction (J) and diversity (D) regions, the contribution of the V (D) J rearrangement to its immunogenetic diversity is small, due to the fact that only a single *locus* undergoes rearrangement [24]. Thus, the genetic diversity of IgY is guaranteed by gene hyperconversion, a process in which pseudogenes donate homologous sequences to the functional gene (V) after V (D) J rearrangement [25].

Even though it is a protein molecule, IgY is resistant to heat and pH, being stable between 30° and 70 °C and active between pH 3.5 and 11. However, the affinity of IgY to its antigen decreases with increasing temperature. Nonetheless, the addition of sucrose, maltose and glycine protects IgY from heat denaturation [8], while sorbitol stabilizes it at acid pH [26]. IgY is resistant to inactivation by the proteolytic enzymes trypsin and chymotrypsin, but is degraded by pepsin [27]. The digestion of IgY with papain leads to a Fc fragment plus two monovalent Fab fragments, like IgG. Nonetheless, IgY digestion with pepsin leads to a pFc′ fragment and two monovalent Fab fragments, unlike mammalian antibodies, which forms one bivalent Fab fragment when cleaved by pepsin [28]. The characteristics of IgY, in comparison to IgG, are summarized in Table 1.

Several methods have been developed to protect IgY from degradation by pH and pepsin present in the stomach, allowing it to arrive unscathed at the small intestine, which is especially important for the use of IgY against enteric pathogens. Among these treatments, chitosan and alginate microcapsules [29], β-cyclodextrin microcapsules [30,31], copolymers of methacrylic acid [32], liposomes [31,33], multiple emulsification [34], hydrogel [35] and carbon nanotubes with hydrogel

Table 1
Comparison of IgY and IgG.

	IgY	IgG
Species	Birds, reptiles, amphibians and lungfish	Mammals
Source	Serum and eggs	Serum
Concentration	100–150 mg/egg (15 mL/yolk)	200 mg/bleed (40 mL)
Molecular weight (kDa)	180	150
Constant domains (Heavy chain)	4	3
Hinge region	No	Yes
pH stability	3.5–11.0	2.0–11.0
Heat stability	Up to 70 °C	Up to 75°–80°
Proteolytic degradation	Pepsin and papain	Pepsin, papain, trypsin and chymotrypsin
Complement binding	No	Yes
Rheumatoid factor binding	No	Yes
Fc receptor binding	No	Yes
Binding to protein A and G	No	Yes

[36] have been tested with different degrees of success. Nonetheless, unprotected IgY consumed as egg yolk in liquid [37] and powder forms [38] showed resistance to the gastrointestinal tract conditions in calves.

3. Production of IgY

The antibody titers are influenced by several factors, such as the antigen type and dose, the used adjuvant, the route of application, the inoculation frequency, age and stage of development in birds [39,40]. Several protocols for obtaining IgY, observing these different variables, have been applied [1,39]. Basically, multiple immunization protocols were tested for different antigens and animals, in order to obtain the highest antibody titer for each case [1].

Several antigen types have been used to produce specific IgY in birds, such as complex antigens (virus, bacteria and parasites) and single antigens (proteins, polysaccharides, peptides and nucleic acids) [40]. Different antigen concentrations can also be combined with adjuvant. However, it is common to use 10 to 100 μg of antigen per mL injected in two or three sites, with the age of hen ranging between seven and eight weeks [1].

The induction of high antibodies titers depends on the use of adjuvants. Though Freund's Complete Adjuvant (FCA) is the most potent to induce antibodies in laboratory animals, it can lead to severe inflammation at the injection site [40]. Although some studies have shown that the immunization with FCA is more tolerated by birds than by mammals and seems to not generate tissue damage in chickens [23,41], other studies using FCA for bird immunization contradict these results [42,43]. Since there is no consensus, one of the best substitutes for FCA is Freund's Incomplete Adjuvant (FIA), which, unlike FCA, does not contain mycobacteria. Shade et al. recommended the use of FIA, which can be used since the first immunization without any prejudice to the antibody titer [3]. In spite of this, the first inoculation is generally performed using FCA, while the subsequent inoculations are performed using FIA, without the occurrence of inflammation at the injection site [44,45].

The most common route of antigen administration for IgY production in chickens is the intramuscular route, where the inoculation is usually into the breast muscle. This technique is most suited for young hens, because subcutaneous injection into the neck is more difficult to perform and may cause more distress to the animal. The intramuscular injection into the leg must be avoided as it can lead to lameness [3]. Nonetheless, for a non-invasive method, the administration of antigen can also be performed orally [46]. The number of inoculations required depends on the type and dose of the antigen, as well as the adjuvant used. At least two inoculations must be performed before the laying period, with an interval of four to six weeks. The titer of IgY must be assessed 14 days after the last immunization. If the antibody titer decreases, further immunizations must be done during the entire laying period to increase antibody titers year round [3]. An increase in titer of egg yolk antibodies can be observed from the second week [47,48] or sometimes from the fifth week post antigen inoculation [49]. After a steady increase, stabilization of the antibody titer occurs, reaching a plateau, and from there on it decreases progressively [48]. Through booster inoculations, it is possible to keep high titers of egg yolk antibodies for more than 150 days [50].

The IgY extraction consists of the removal of lipids to form a water soluble fraction (WSF), followed by the precipitation of the antibodies contained therein. Several extraction methods are available for obtaining IgY from egg yolk and the choice of the suitable method depends on the purpose, which can require different purification degrees, as well as the extraction scale, cost and available technology [40].

A frequently used method for the extraction of IgY is the precipitation with polyethylene glycol (PEG) 6.000. The method consists on the delipidation by centrifuging egg yolk diluted in phosphate buffered saline (PBS) with 3.5% of PEG 6000. Next, the supernatant is twice centrifuged with 12% of PEG 6000 to precipitate the antibodies [6]. Akita & Nakai obtained highly purified IgY by removing the lipids from the yolks by means of six-fold dilution in water at pH 5.0, with an incubation time of 6 h at 4 °C, followed by IgY precipitation with 60% of ammonium sulfate $(NH_4)_2SO_4$, which was supplemented by the addition of sodium chloride (NaCl) or by ultrafiltration prior to gel filtration or ion exchange chromatography. The authors also achieved highly purified IgY by means of ethanol precipitation at lower temperatures [51]. Nonetheless, a study conducted by Araújo et al. showed that the most suited concentration of $(NH_4)_2SO_4$ for IgY's precipitation is 20%. In this study, higher concentrations of $(NH_4)_2SO_4$ also caused the precipitation of non-specific proteins, while a lower concentration led to a greater loss of antibodies in the supernatant [52].

Since the Fc region of IgY does not bind to proteins A and G, which are commonly used in the affinity purification of mammalian IgG, affinity chromatographic methods for IgY purification requires other types of ligands. IgY can be purified through the adsorption of the immunizing antigen to the solid phase of the affinity column, which generates monospecific antibodies that can be eluted under acid [53] or alkaline conditions [54]. The purification of IgY can also be performed through thiophilic adsorption chromatography [55]. In contrast to other affinity purification methods, it can be performed at mild conditions [56].

Hens are able to produce between 100 and 150 mg of IgY antibodies by yolk, of which between 1 and 10% are specific [1]. In one year, a single laying hen produces between 17 and 35 g of total IgY [13]. Although the production of IgY for research is mainly done in chicken, other birds are also used for the same purpose, such as goose [57] and quail [58], using an immunization protocol similar to that used for chickens.

The production of IgY and its possible applications in both human and veterinary health are summarized in Fig. 2.

4. Therapeutic and prophylactic applications of IgY

4.1. Antibacterial activity

The use of polyclonal IgY against infectious diseases minimizes the risk of microbial resistance, since the antibody is directed to various antigens of the same microorganism, which requires multiple genes for its synthesis [8]. Therefore, specific IgY antibodies are a relevant alternative to use as antimicrobials in human and veterinary health in the face of the emergence of resistant bacteria. IgY antibacterial activity can be assessed by the inhibition of bacterial growth and biofilm formation *in vitro*, as well as adhesion ability, since they are prior conditions for successful colonization of a higher animal by bacteria and viruses [13]. IgY antibacterial activity against gastrointestinal pathogens has been widely investigated. Nasiri et al. extracted IgY from hens immunized with the recombinant protein FanC, from enterotoxigenic *Escherichia coli* (ETEC) and these antibodies bound specifically to FanC in ELISA, Western blot and Dot-blotting [59], demanding, thus, more investigations to evaluate its viability as a potential immunotherapeutic compound.

Li et al. evaluated the effect of specific and non-specific IgY against *Salmonella typhimurium* on the immune response of mice infected by the bacterium [60]. Specific IgY reduced the expression of the pro-inflammatory cytokines TNF-α and INF-γ and elevated that of the anti-inflammatory cytokine IL-10; while non-specific IgY also reduced the level of TNF-α, but did not alter the expression level of INF-γ and IL-10. Although both specific and non-specific antibodies reduced the number of T lymphocytes in the *lamina propria* and of $CD4^+$, $CD8^+$ and γδ T lymphocytes in the jejunum of mice, the animals treated with anti-*S. typhimurium* IgY had a longer survival rate than those treated with non-specific IgY.

IgY activity against bacterial spores was also investigated. Oral administration of IgY against *Clostridium difficile* spores both delayed the onset of diarrhea in rats treated prior to the infection and reduced

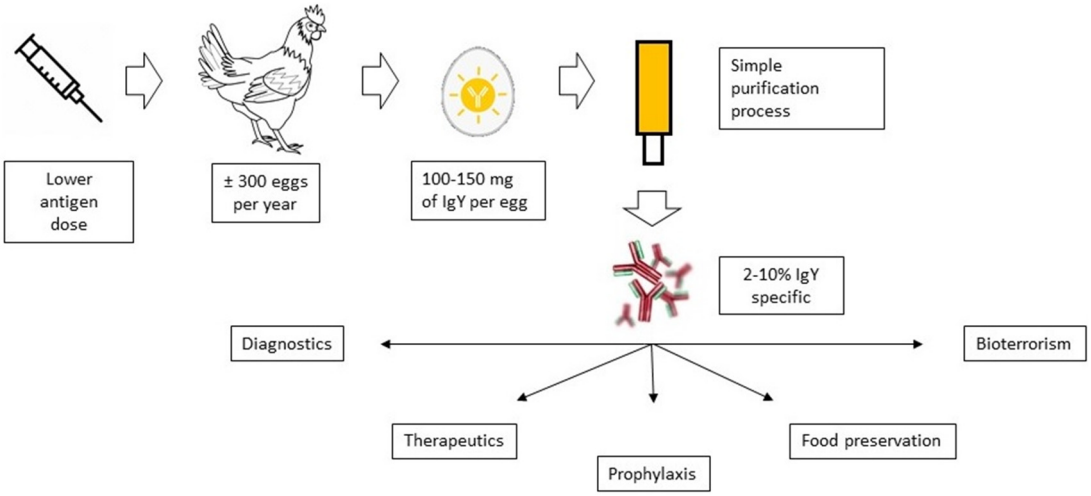

Fig. 2. Production and applications of IgY.

its recurrence in infected rats. Thus, specific IgY could be used in the prophylaxis of *C. difficile* infection, as well as an immunotherapeutic to eliminate the remaining spores on the intestines of people who have already had the infection, which would reduce the recurrence of diarrhea [61].

The therapeutic action of IgY against *Helicobacter pylori* has been largely studied as well, through antibodies directed to both the whole cell and to specific proteins. Oral administration of Anti-HpUc (*H. pylori* urease) IgY inhibited the growth of *H. pylori in vitro* and reduced the inflammation of stomach in mice, with a significant reduction of neutrophils and lymphocytes infiltration on the tissue. A decrease in the serum level of antibodies against *H. pylori* was also observed in treated animals [62]. Similar results, as the reduction of serum levels of anti-*H. pylori* IgG and the absence of inflammation signs on the stomach were observed in mice that received oral IgY against the *H. pylori* vacuolating cytokine A (VacA) [63].

Specific IgY against HP-Nap protein, the main virulence factor of *H. pylori*, significantly inhibited the adhesion of the bacterium to an AGS cells culture [64]. Solhi et al. evaluated the occurrence of cross reaction between strain-specific IgY and four different *H. pylori* strains. The antibodies inhibited the cell growth and the urease enzyme not only of the strains for which they were specific, but also of other strains [65].

The therapeutic potential of IgY against bacterial respiratory tract infections has also been considered. IgY against *Mycobacterium tuberculosis* increased the proliferation of PBMC (peripheral blood mononuclear cells) in rats in a dose-dependent manner. An increased expression of cytokines that stimulate the cellular immune response was observed and an increase of mRNA level of IL-2 and IFN-γ was detected by RT-PCR, which indicates that anti-*M. tuberculosis* IgY is a potential immunotherapeutic that stimulates T-helper 1 immune response [66].

Shi et al. produced specific IgY against multi-drug resistant strains of *Acinetobacter baumannii*. The antibodies inhibited bacterial growth *in vitro*, significantly reduced the mortality of mice infected with the bacterium and attenuated the lung inflammation [67].

IgY has also been tested as a potential therapeutic resource in oral infections against *Prevotella intermedia*. Specific IgY inhibited bacterial growth in liquid medium and showed therapeutic activity in rats with *P. intermedia* induced gingivitis. The reduction of gingival inflammation, bacterial plaque and bleeding, as well as the normalization of WBC (White Blood Cells) levels in the blood, were observed in treated animals. Histopathological examination showed no signs of inflammation in the gums of rats treated with anti-*P. intermedia* IgY [68].

Additionally, studies have shown inhibitory growth effect of therapeutic IgY produced against *Fusobacterium nucleatum* in liquid medium and in the formation of biofilm in polyestyrene plates. Anti-*F. nucleatum* IgY also reduced bone loss in mice with periodontal disease when applied after the infection, thus presenting therapeutic function [69].

The therapeutic effect of IgY against acne was also evaluated. Revathy et al. produced and assessed the efficiency of specific IgY against the bacterium *Propionibacterium acnes*. The antibody inhibited bacterial growth and biofilm formation *in vitro*, proving to be a possible alternative to antibiotics in the treatment of acne [70].

More recently, IgY was tested for antibacterial effect in aquatic animals. Gao et al. produced IgY against *Vibrio* spp., the major cause of death in white shrimp (*Litopenaeus vannamei*), and demonstrated that powder egg yolk containing anti-*Vibrio* IgY significantly reduced the mortality of white shrimp infected with *V. harveyi* and *V. parahaemolyticus* [30], which indicates that therapeutic IgY can also be applied in aquafarming.

The prophylactic potential of IgY against dental caries has been widely studied. An interesting study carried by Bachtiar et al. evaluated the effect of soybean milk containing IgY against the cariogenic bacterium *Streptococcus mutans* in rats. The animals fed with this milk presented a decrease in the number of *S. mutans* in dental biofilm and fifteen days after milk intake IgY could still be detected in the saliva [71]. In other research, the effect of specific IgY against the cell-associated glucosyltransferase enzyme (CA-gtf), of *S. mutans*, was evaluated. As a result, Anti-CA-gtf IgY inhibited bacterial adhesion *in vitro* and suppressed the oral cavity colonization [72].

In addition, specific IgY action against the ComD protein, a *quorum-sensing* signals receptor of *S. mutans*, was also explored. It inhibited, *in vitro*, the development of biofilm of *S. mutans* from the oral cavity of people with and without caries, and changes in the protein expression pattern were found in the bacteria treated with anti-ComD IgY [73].

The protective effect of IgY against opportunistic infections was also investigated by Thomsen et al., where, by means of chemiluminescence, the production of reactive oxygen species (ROS) in polymorphonuclear neutrophils (PMN) was evaluated as an indicator of the intensity of PMN mediated immune response against *Pseudomonas aeruginosas in vitro*, as well as the influence of anti-*P. aeruginosas* IgY in this process. The specific IgY opsonized the bacterium and intensified the cellular immune response, probably due to the recognition of IgY by receptors resembling avian IgY receptors or physical-chemical changes in the bacterium, since IgY does not activate mammalian Fc receptors. Anti-*P. aeruginosas* IgY could be used in the prophylaxis of *P. aeruginosas* infection in patients with Cystic fibrosis by increasing the cellular immune response [74].

Prophylaxis of bacterial infections in animals for consumption, using IgY, was also considered. Specific IgY incorporated into hydrogel

added to carbon nanotubes and chitosan (H-CNT) showed protective activity against enterotoxigenic *Escherichia coli* (ETEC) in piglets [75]. Thus, Anti-ETEC IgY incorporated into H-CNT and orally applied could be used to prevent the intestinal infection in these animals.

In order to generate antibodies against the bacterium *Campylobacter jejuni* to be used as food additive aiming to control *C. jejuni* colonization in chickens, Thibodeau et al. produced anti-*C. jejuni* IgY in three different ways: oral administration of a pool of living bacteria from four *C. jejuni* strains, subcutaneous injection of extracts from the outer membrane of the bacterium or of formalin inactivated bacterium. The hens submitted to subcutaneous inoculation produced IgY more intensely, although the antibodies obtained through oral inoculation also showed good activity. Anti-*C. jejuni* IgY, obtained from the three ways, presented bactericidal activity *in vitro* [46].

IgY against the bacterium *Aeromonas salmonicida*, the causative agent of ulcers on fish skin, was added, in powder form, to the water in which Koi carp (*Cyprinus carpio koi*) ornamental fish were raised. Anti-*A. salmonicida* antibodies showed good prophylactic activity against the infection, and a small amount of specific IgY was enough to completely prevent the appearance of skin ulcers in healthy fish that cohabited with sick fish previously infected with *A. salmonicida* [76].

The potential of IgY technology to prevent bacterial infections in animals could be useful in the needful strategies to reduce the use of antibiotics in food animal husbandry, which has been linked to the selection and spread of resistant bacterial strains that threaten antibiotics as a therapeutic resource [77,78].

4.2. Antiviral activity

The therapeutic potential of IgY against viral infections of the gastrointestinal tract in animals has been extensively investigated. Oral administration of egg yolk powder rich in IgY against bovine group A Rotavirus (RVA) was performed on calves infected by the virus. As a result, disease attenuation occurred in treated animals, with a reduction of the period of diarrhea and hyperthermia and the absence of other symptoms observed in untreated calves, such as anorexia, dehydration and depression. The egg yolk powder enriched with anti-RVA IgY retained the activity after two years, when kept at 4 °C [38]. In another study, IgY against the S1 protein of porcine epidemical diarrhea virus (PEDV) was produced and orally administered in piglets previously infected with PEDV. Anti-S1 IgY reduced the severity of diarrhea and intestinal lesions caused by PEDV infection and nullified the mortality of piglets due to the disease [79].

Another possible therapeutic use of specific IgY is against dengue fever, as demonstrated by Fink et al. Anti-DENV2 IgY produced in goose was able to neutralize the virus *in vitro* and *in vivo* without binding to Fcγ receptors on myeloid cells and generating ADE (antibody dependent enhancement) in mice [57].

The protective effect of IgY against influenza has been widely studied. IgY against the avian influenza A virus (H5N1) were extracted from eggs available in supermarkets of Vietnam, where the vaccination of chickens against H5N1 virus is required. Anti-H5N1 IgY was administrated intranasally in mice before and after their infection with H5N1 and H5N2 and completely prevented the disease onset [80]. These results reveal that commercially available eggs that are produced in countries where anti-H5N1 vaccination is mandatory constitute a considerable source of IgY that could be used to prevent a potential pandemic of H5N1 virus.

Following this rationale, Wallach et al. produced IgY against H1N1, H3N2 and H5N1 strains of influenza virus, which were tested for their ability to neutralize homologous and heterologous strains *in vitro* and to prevent the infection *in vivo*. The antibodies inhibited only homologous strains in the seroneutralization and in the haemagglutination inhibition assay, except anti-H5N1 IgY, which also inhibited H1N1. Anti-H5N1 and anti-H1N1 IgY were applied intranasally in mice 1 h before the infection by influenza virus and showed prophylactic activity. Thus, IgY against influenza virus strains could be used in nasal, oral or spray applications to protect individuals and environments [81]. IgY against influenza B virus (IBV) was also tested and neutralized the activity of haemagglutinins and neuraminidase present in the virus and inhibited viral replication *in vitro*. When applied intranasally, anti-IBV IgY prevented influenza development in mice treated prior to virus exposure and attenuated the disease in those treated post-infection [48].

The prophylactic effect of IgY against other viral infections of the respiratory tract was also evaluated. IgY against Andes virus (ANDV), the causative agent of the Hantavirus Pulmonary Syndrome (HPS), was obtained from geese eggs by inoculating these animals with the DNA encoding the virus envelope glycoprotein. Anti-ANDV IgY was able to prevent HPS development in recently infected mice but failed when applied after the onset of viremia, thus presenting prophylactic and non-therapeutic activity [82].

The protective effects of IgY were also considered for viral infections in poultry. Aizenshtein et al. produced, in the same eggs, efficient IgY against the Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), influenza and reovirus, which are pathogenic for birds [83], demonstrating that passive immunization with IgY against several viruses is possible; nonetheless, it is a technology that must be further explored.

4.3. Antifungal activity

A gel preparation for oral use containing IgY against *Candida albicans* was tested by Tekeuchi et al. and caused a reduction in the number of colony-forming units (CFU) on the oral cavity of elderly people, showing promise for prophylactic use against *C. albicans* oral infection [84]. In other research, specific IgY inhibited the adhesion of *Candida albicans* and *Candida glabrata* to denture base material. Anti-*C. albicans* IgY was more effective against *C. albicans* than anti-*C. glabrata* IgY, while both antibodies were equally effective in preventing the adhesion of *C. glabrata* [85].

4.4. Antiparasitic activity

Sampaio et al. produced a high avidity IgY against the protozoan *Trypanosoma evansi*. There was neither prophylactic action nor infection control in the treated rats, but anti-*T. evansi* IgY increased their survival rate when used concomitantly to anti-*hematozoa* drugs [86]. In another study, Grando et al. inoculated *Trypanosoma cruzi* trypomastigots in hens and extracted a high amount of anti-*T. cruzi* IgY that cross-reacted with *T. evansi* antigens in Western blot, showing that the anti-*T. cruzi* IgY is not reliable as a diagnostic tool, but deserves more investigations as a possible therapeutic resource for trypanosomes infection [5].

4.5. Antitumor activity

The phylogenetic distance between birds and mammals ensures a stronger immune response against mammalian antigens by birds [23]. Such a feature may be advantageous to produce IgY against human tumor antigens. Following this rationale, Amirijavidv et al. produced highly specific IgY against a sequence of 21 amino acids present on the ectodomain of the TRAIL (TNF-related apoptosis-inducing ligand) receptor TRAIL-R2 (DR5). The antibodies bound to the amino acid sequence and activated the DR5 receptors in human breast cancer cells MCF7, acting as a TRAIL agonist and inducing apoptosis [87]. IgY against other receptors, such as the HER2 receptor, was tested coupled to single walled carbon nanotubes (SWNTs) and specifically detected the HER2 receptors on the surface of SK-BR-3 cells. The binding of the complex to the receptors was measured by Raman signals emitted by the nanotubes. SWNT has a near infrared absorption (NIR), which can be used for tumor ablation, and, coupled to anti-HER2 IgY, was able to kill SK-BR-3 cells without needing internalization of the complex by the cell [88]. These findings show that IgY produced against tumor antigens

is an attractive alternative for a more selective treatment of cancers and its use could, therefore, minimize the side effects of traditional chemotherapy.

4.6. Antiobesity activity

IgY raised against porcine pancreatic lipase was used against the enzyme *in vitro* and *in vivo*. Later, mice with obesity induced by high fat diet were orally treated with the antibody, which was given concomitantly with food, and a reduction of adipose tissue and liver fat level was observed, as well as an increase of fecal excretion of triglycerides and their decrease in blood plasma. Anti-lipase IgY inhibited the hydrolysis of diet fat and reduced its intestinal absorption, showing anti-obesity activity [89].

4.7. Antiallergic activity

Wei-xu et al. evaluated the antiallergic effect of specific IgY against the pro-inflammatory cytokines IL-β1 and TNF-α in guinea pigs with induced allergic rhinitis. A reduction of the eosinophils number in the blood and in the nasal and bronchial lavages was found, as well as a decrease of eosinophils, neutrophils and lymphocytes infiltration into the nasal mucosa and the lungs of animals treated with anti-IL-β1 and anti-TNF-α IgY, alone or jointly [90].

4.8. Anti-venom activity

One of the side effects that occur in individuals receiving anti-venom serum produced in goats, sheep and horses is due to the presence of serum proteins on the anti-venom serum derived from these animals, in which IgG is not sufficiently purified [52,91]. One advantage of using IgY in anti-venom serotherapy is that it is easily purified, which would minimize the occurrence of side effects due to non-specific proteins. Araujo et al. demonstrated this property when specific IgY was produced as anti-venom of the snake genus *Bothrops* sp. These antibodies neutralized a pool of venoms from five *Bothrops* species, with an ED50 of 150 μL/2LD50, showing little to no side effects in mice [52].

Mendonza et al. also produced IgY capable of neutralizing the venom of the peruvian snake *Bothrops atox*. The anti-venom IgY showed considerable cross reaction with the venom of *Bothrops brazili* and could be used not only as *B. atox* anti-venom, but also as a tool for the research of cross reaction with venoms from different species [9].

In another elegant work, Andrade et al. produced IgY against a pool of venoms from snakes of the genus *Bothrops* and against the venom from the species *Crotalus durissus terrificus*. Anti-venom IgY extracted from eggs was compared to the horse anti-venom IgG in Western blot. The results showed that specific egg's IgY recognized the same antigens as the equine anti-venom [92].

IgY against coral snake venom was first produced in response to a pool of venoms from different species of *Micrurus*. These antibodies recognized, by Western blot, venom proteins from several snakes: *M. isozonus*, *M. surinamensis*, *M. f. fulvius*, *Naja kaouthia*, *N. pallida*, *Bothrops colombiensis*, *Crotalus durissus cumanensis* and *C. vegrandis* and could, therefore, be used as a broad-spectrum snake anti-venom [93].

Zolfagharian and Dounighi produced IgY by inoculating the *Vipera lebetina* snake venom, inactivated by γ radiation, in hens [94]. These antibodies were effective in neutralizing the crude venom of *Vipera lebetina* in mice.

Anti-venom IgY were also obtained from eggs of hens immunized with the venom of the snake *Trimeresurus albolabris*. These IgY recognized, by Western blot, most of the proteins present in the *T. albolabris* venom and neutralized it in mice in a dose-dependent manner [95].

More recently, Liu et al. extracted and purified IgY from eggs of hens inoculated with the venom of the *Deinagkistrodon actus* snake. These antibodies were able to neutralize the lethal effects of the venom, such as bleeding, edema formation and myotoxicity in a dose-dependent manner [96].

Da Rocha et al. produced IgY against ophidian toxins of *Crotalus durissus terrificus*, *Bothrops jararaca* and *Bitis arietans*. The antibodies were able to bind to specific components of the venoms in Western blot and protected 100% of the intoxicated mice when obtained after the ninth inoculation. The authors recommended the use of a small antigen dose (20 μL) applied in successive inoculations for IgY production, since this dose was enough to genetically alter the V(D)J segments on the naïve cells and to generate immunological memory [97].

However, IgY raised against the venom of the snake *Oxyuranus scutellatus* was less effective than equine IgG, being unable to neutralize the neurotoxic and coagulant effects of the venom [98]. Nonetheless, this result cannot be extended to IgY produced against other venoms.

IgY against the *Tityus caripitensis* scorpion venom, produced by Alvarez et al., neutralized not only the venom of *T. caripitensis*, but also that of other *Tityus* species (*T. quirogae*, *T. discrepans* and *T. gonzalespongai*), and inactivated the hyaluronidase, an enzyme that facilitates the toxin spread in the tissues, present in the *T. serrulatus* venom [99]. Thus, IgY raised against *T. caripitensis* venom could be used as a broad-spectrum anti-scorpionic serum.

4.9. Prophylaxis of Celiac disease

Another application of IgY technology, the prophylaxis of Celiac disease, was demonstrated by Gujral et al., who developed powdered egg yolk formula with protective sugars containing anti-gliadin IgY, among which, the formula with mannitol (EYP-M) retained its activity after being submitted, *in vitro*, to chemical conditions analogous to those of the stomach and small intestine. The formula IgY-EPY-M neutralized *in vitro* both the isolated gliadin and that present in food matrix and inhibited its intestinal absorption in mice, showing promising for the prevention of Celiac disease [100].

4.10. Prophylaxis of toxicity

Bobeck et al. used IgY against the human intestinal alkaline phosphatase (hIAP) to assess the influence of IAP on increased bioavailability of phytate phosphate in the presence of 1α-dihydroxycholecalciferol (vitamin D3) in chickens. Anti-hIAP IgY was ingested by chickens and reduced the absorption of phytate phosphate, which suggests that although it performed less adequately than sevelamer chorhydrate, already used for the same purpose, anti-hIAP IgY can be optimized for the prevention of phytate phosphate toxicity induced by the consumption of the active form of vitamin D [101].

5. Applications of IgY in diagnosis

5.1. Viral infections diagnosis

The ability of IgY to detect viral pathogens of the gastrointestinal tract in humans and animals has been widely studied. IgY raised against canine parvovirus viral like particles (CPV-VLPs) were used in ELISA and immunochromatography, showing sensitivity and specificity in the detection of canine parvovirus in dog fecal samples [102].

Specific IgY against the E2 protein of bovine viral diarrhea virus (BVDV) were used in ELISA to detect pathogens that cause diarrhea in cattle. Anti-E2 IgY showed a high specificity, recognizing only BVDV. ELISA and immunochromatography tests using these antibodies were efficient in detecting BVDV in serum samples of cows with diarrhea, showing a concordance of 95, 45% and 90% with RT-PCR, respectively [103].

Da Silva et al. used IgY against hepatitis A virus (HAV) in a competitive immunoenzymatic assay to detect anti-HAV IgG in serum samples, showing satisfactory sensitivity and specificity [104]. More recently, IgY against HAV was used to detect the virus in hepatic

sections of infected *rhesus* monkeys by means of indirect immunofluorescence (IIF). Anti-HVA IgY was more efficient than the commercially available IgG for the detection of the same antigen [105].

IgY was also used to detect viral pathogens in aquatic animals. Specific IgY against the soft-shelled turtle systemic septicemia spherical virus (STSSSV) was used to compose a lateral flow assay to detect the virus in turtles. This assay was sensitive and specific, detecting STSSSV in all infected turtles in serum and feces samples [106].

A potential for diagnosis was presented by IgY raised against the nucleocapsid protein (NP) of coronavirus (CoV). Anti-NP IgY, used as capture antibody in ELISA for detecting NP, lowered its detection limit to a picogram level, which indicates that the antibody is promising for use in the diagnosis of acute respiratory syndrome associated to coronavirus (SARS-CoV) and is sensitive enough to detect small amounts of NP [107].

Moreover, the ability of IgY in diagnosing dengue fever was also evaluated. Figueiredo et al. produced IgY against the non-structural protein 1 (NS1) of dengue virus 2 (DENV2). These antibodies were used to compose an immunosensor that was effective in electrically detecting the NS1 protein of DENV2 in standard samples and could be used for dengue 2 diagnosis in biological samples [108].

5.2. Bacterial infections diagnosis

One of the most studied diagnostic potential of IgY is against *Staphylococcus aureus*. The fact that the Fc portion of IgG reacts with the staphylococcal protein A makes IgY a relevant resource for more specific detection of different *S. aureus* strains and their toxins, since, due to structural differences, the Fc portion of IgY does not react with protein A [109].

Following this rationale, Walczak et al. produced IgY against the fibrinogen binding protein (Efb) of *Staphylococcus aureus* and against a peptide epitope that encompasses the residues 127–140 of Efb protein. Anti-Efb and anti-$Efb_{127–140}$ antibodies presented high titers in ELISA and strong avidity in Western blot, showing promising use in the diagnosis of *S. aureus* infection [110].

Another ELISA test using IgY against the staphylococcal enterotoxin B (SEB) of *S. aureus* as capture antibody and specific ssDNA aptamers coupled to biotin as revealing was developed by Mulidi et al. This assay was efficient in detecting SEB, but also reacted with others staphylococcal toxins, such as staphylococcal endotoxins A (SEA) and C (SEC), toxic shock syndrome toxin (TSST) and α-hemolysin [111].

IgY raised against α-hemolysin was applied as capture antibody in ELISA for detecting the toxin in the supernatant of *S. aureus* cultures. Anti-αhA IgY showed high specificity against the toxin, without reacting with protein A, which is present in all *S. aureus* strains [44].

The ability of IgY to diagnose resistant *S. aureus* was also investigated. Yamada et al. produced IgY against the penicillin binding protein (PBP) 2a, which is present in methicillin resistant *S. aureus* (MRSA) strains. Anti-PBP2a IgY was used in ELISA, lateral flow and latex to detect MRSA and other *S. aureus* strains sensitive to methicillin and beta-lactams. The antibody was MRSA specific and did not react with the sensitive strains that express significant amounts of protein A [112].

5.3. Parasitic infections diagnosis

Among the parasitic infections tested, Cakir-Koc evaluated the potential of IgY in detecting the protozoan *Toxoplasma gondii* by producing IgY against its surface protein SAG-1, which reacted with the target antigen in ELISA and Western blot [113]. Therefore, this study revealed a potential diagnostic test for toxoplasmosis. More recently, Anti-SAG1 IgY conjugated to fluorescein isothiocyanate detected *T. gondii* tachyzoites in a colony, by means of immunofluorescence, and may be used to detect the protozoan in other types of samples [114].

In other interesting research, an indirect ELISA using IgY to detect cathepsin F (*CF*), a cysteine protease present in the helminth *Opisthorchirs viverrini,* was developed. This assay showed good sensitivity in the detection of *O. viverrini* in fecal samples from humans living in endemics places; however, a cross reaction with *Taenia* spp., *Echinostoma* spp. and *Minute Intestinal Fluke* (MIF) was observed [115]. Therefore, improvements still have to be made before this technology is available.

Miura et al. produced IgY against the GP60 protein, from the *Cryptosporidium hominis* protozoan. These IgY bound to GP60 in Western blot and to *C. parvum* sporozoites in fecal samples by indirect immunofluorescence, suggesting that anti-GP60 IgY could be used in the diagnosis of cryptosporidiosis caused by both *C. hominis* and *C. parvum* [116].

5.4. Diagnosis of tumors

Several authors have been investigating the potential of IgY in the detection of tumor markers. IgY against the peptide antigen CA 15-3, a commonly used breast cancer marker, was used as secondary antibody in a sandwich ELISA aiming to detect CA 15-3, showing potential for clinical use [49]. Sun et al. produced IgY against two portions of the transmembrane glycoprotein HER2: HER2-A, proximal region, and HER2-B, distal region. Anti-HER2-A and anti-HER2-B IgY effectively detected the HER2 glycoprotein in cultured breast cancer SK-BR-3 cells by immunofluorescence and in sections of breast tumors over expressing HER2-B, using immunohistochemistry. In addition, the antibodies bound to the glycoprotein in ELISA and Western blot, which indicates that IgY against HER2 is promising for use in breast cancer diagnosis [117].

In another study, Łupicka-Słowik et al. developed a direct ELISA test using a lysate of human malignant tumor cells and IgY against bovine adenosine deaminase (c-ADA). The assay was efficient in detecting human adenosine deaminase (h-ADA) present in the tumor cells lysate, which was possible due to the high homology between c-ADA and h-ADA. The ELISA test using anti-c-ADA IgY could be used to diagnose several types of malignant tumors in humans, as well as be an alternative to currently employed enzymatic methods for the detection and quantification of ADA for pleural tuberculosis diagnosis [118].

Another IgY developed to detect prostatic specific antigen (PSA) and two peptide fragments of this protein demonstrated specificity and marked the antigen more strongly than the IgG counterpart using Western blot analysis. However, anti-PSA IgY had an unsatisfactory sensitivity when applied as secondary antibody in indirect ELISA [45], thus deserving more investigation.

In general, these findings suggest that the phylogenetic distance between birds and mammals, that ensures a stronger immune response against mammalian antigens by birds than by other mammals [23], makes the production of IgY against tumor antigens advantageous not only for therapeutic purposes, as described above, but also for usage in several types of immunoassays for tumor detection in humans.

5.5. Hematological tests

Hens immunized with umbilical cord sera produced specific IgY against IgG and the complement fractions C3b and C3d. These antibodies did not react with the C4b fraction - which configures a higher specificity, since anti-C4b antibodies often cross-reacts with the antigens of Chido/Rodgers RBC group - nor with erythrocyte antigens from ABO group. These antibodies are, therefore, promising as a reagent for Coombs test [119].

5.6. Enzyme detection

IgY immunoassays were used to detect the hepatic expression of Cytochrome P450 2E1 (CYP2E1) in mice treated with medicinal herbs and products derived from plants rich in flavonoids. CYP2E1

metabolizes a wide variety of chemicals with different structures, in particular small and hydrophobic compounds, including potential cytotoxic and carcinogenic agents. Anti-CYP2E1 IgY was specific, without reacting with other cytochromes, and was able to detect the reduction of hepatic CYPE2E1 expression due to the ingestion of natural products with hepatoprotective effects [120].

5.7. Identification of substances

The ability of IgY in identifying harmful substances in consumer products has been evaluated. An ELISA test was developed to detect the staphylococcal enterotoxin G (SEG), using specific IgY, and showed satisfactory sensitivity and specificity, reducing the interference of protein A that occurs in IgG tests. This test was successfully used to detect SEG in milk and dairy products samples and could therefore be used to identify the toxin in food [121].

Bittner et al. used IgY in ELISA to detect potentially allergenic proteins in commercially available latex gloves. This assay presented similar results to that of the gold standard test, which uses mammalian IgG [122].

IgY can also be used in assays to identify antibiotic residues in food products of animal origin, as demonstrated in a study by He et al., in which produced anti-gentamycin IgY specifically detected the target antibiotic present in animal origin products [123]. Following this rationale, Li et al. used specific IgY to detect kanamycin and gentamycin residues in milk and meat samples by means of competition ELISA and FPIA (fluorescence polarization immunoassay) [124].

The potential of IgY in identifying substances has also been used to evaluate the toxicity of a natural product employed in the alternative medicine. IgY labeled with Quantum dot were successfully used in a lateral flow assay for the detection of rhein; a toxic substance found in the plant *Rheum officinale*, widely used in Chinese traditional medicine; in plant samples and serum from users [125].

6. Other important applications

6.1. Food preservation

IgY raised against the bacterium *Listeria monocytogenes* showed a significant inhibitory effect of bacterial growth in liquid medium and in fish samples stored between 0 and 6 °C in a dose-dependent manner, which indicates that anti-*L. monocytogenes* IgY is a potential antimicrobial for use in the food industry [47]. Taking into consideration the versatility and the range of IgY already tested against several bacteria, this result could be easily apply to other food poisoning bacteria and viruses.

6.2. Use of IgY in bioterrorism circumstance

Among the importance of this technology, LeClair et al. demonstrated that IgY produced against the staphylococcal enterotoxin B (SEB), a potential biological weapon, can save individuals exposed to this material. The results with *Rhesus* monkeys showed that animals that received anti-SEB IgY 30 min before or 4 h after exposure to a lethal SEB aerosol survived [10], which indicates that anti-SEB IgY could be used to protect populations in a hypothetical context of bioterrorism involving SEB [13].

7. Conclusion

The latest findings using IgY have clearly demonstrated the versatility of this technology. Obtaining IgY from birds presents several technical and economical advantages over mammalian IgG, and as described in this review, IgY technology has a broad spectrum of applications in human and veterinary health. It can be used in multiple types of therapies; it is useful in the prevention of various types of diseases and detects, by means of different techniques, several classes of antigens, such as microorganisms, tumor markers and substances.

Among the advantages of this technology, the replacement of invasive antibody collection by its extraction from eggs is one of the most interesting, considering the animal welfare benefits, with this technology it is possible to achieve great quantities of antibodies with a lower cost of production and less damage to animal welfare.

Due to its structural differences and phylogenetic distance, IgY is more specific for diagnostic use and displays greater avidity for mammalian conserved proteins than IgG, being, thus, an important alternative in the search for more effective diagnostics and therapies. In addition, in view of its proven ability to neutralize microorganisms, IgY represents an important therapeutic resource in times of increasing resistance to antibiotics and emergence of viral diseases for which there is no treatment.

Declaration of Competing Interest

There are no funding or competing interests to report.

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