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Utilization of Immunoglobulin Y (IgY) as Alternatives to Laboratory Animals in Human and Veterinary Medical Practice: A Review

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ABSTRACT

Emerging infectious diseases and antibiotic resistance impose major threats to animals and humans throughout the world. The Emergence of various infectious and pandemic diseases alarms the need for prophylactic and therapeutic regimens in protecting both animals and humans. Hence, researchers are trying to identify newer methodologies to overcome the existing issues for better immune response and antibiotic resistance. In this view, chicken immunoglobulin Y (IgY) has gained momentum due to its role in prophylactic, therapeutic and diagnostic modalities with varying success rate. In an unpredictable and contagious COVID-19 like situations, the role of passive immunization through chicken immunoglobulin has turned to be important for controlling infectious disease transmission. This review focus on the immunoprophylactic and immunotherapeutic role of IgY in human and animal diseases and to explore the use of IgY for veterinary and human medicine.

Key words: Chicken immunoglobulin Y, Human, Immunology, Passive, Prophylaxis, Review, Therapeutic, Veterinary practice.

In recent times, emerging, reemerging infectious diseases and antimicrobial drug resistance are the predominant reasons for the failure of treating diseases in individuals. The antimicrobial resistance is one of the potent threats for maintaining global health in the current decade (Asokan and Kasimanickam 2013; Ventola, 2015). Over 100 years of clinical practice, antibiotics are one of the major drugs used in human and animal medical practice with various modes of utility such as prophylactic, therapeutic and growth promoters (Turner *et al*., 2001; Cromwell, 2002; Diraviyam *et al*., 2014). Indiscriminate and injudicious use of antibiotics have resulted in antibiotic resistance among common pathogens with the potential to affect humans and animals. In such circumstances, researchers are currently working on drugs or molecules which are “alternative to antibiotics”, with varying success rate (Asokan and Kasimanickam 2013; Diraviyam *et al*., 2014). Avian immunoglobulin Y (IgY) has recently gained importance in the medical practice with wide applicability such as immunoprophylaxis, immunotherapeutic and immunodiagnostics (Abbas *et al*., 2019; Pereira *et al*., 2019). In mammals, passive immunization of young ones is achieved through the colostrum. Similarly, IgY is the predominant immunoglobulin transferred to chick through egg yolk along with minor portion of IgA and IgM. Presently, IgY are developed against specific target antigen or gene with the various mode of utility. Additionally, non allergic with host immune system and less animal necessity for producing IgY based applications, accelerate the role of IgY in immunological platforms. Based on this fact this review addresses the role of avian immunoglobulin Y (IgY) as immunoprophylaxis and immunotherapeutic for human and animals, through a published literature survey.

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Immunoglobulins

Immunoglobulin or antibodies are the base ground for humoral or antibody mediated immunity. Immunoglobulins are large Y shaped glycoproteins, principally secreted by plasma cells against common pathogens to neutralize them. In all living animals, immunoglobulins are the paramount molecules to fight against infections. The immunoglobulins

are broadly categorized into various types *viz*.,IgM, IgG, IgD, IgE, IgA, IgD2, IgNAR, IgW, IgX, IgY, IgT and HCAbs based on structure, type of chain arrangements and molecular weight. The presence of various immunoglobulins in animals are species specific (Vadnais *et al*., 2017).

Chicken (*Gallus gallus domesticus*) has been utilized in the field of immunology for the purpose or reason out. Hen's immune system differs from mammals in various ways *viz*., the bursa of fabricius is the most important lymphoid organ present in hens but not in mammals. In chicken the predominant immunoglobulin in serum as well as in egg yolk is IgY, whereas in mammals it is IgG and IgE which are absent in the chicken immune system (Kovacs-Nolan and Mine, 2012).

The B cells in chicken are not major histocompatibility complex restricted lymphocytes and hence can capture soluble antigens. Following invasion of an antigen, the B cells begin clonal expansion and then differentiated into plasma cells which are the main source for antibody production (Taebipour *et al.,* 2017). The chicken immune system has mainly three antibody classes; IgM, IgA and IgY, while the mammal's immune system has five antibody classes namely IgM, IgA, IgG, IgE and IgD (Zhang *et al*., 2017). Though structural dissimilarities are present between IgY (avian) and IgG (mammals), the functional features are more similar (Kovacs-Nolan and Mine, 2012). IgY antibody shares structural similarities with mammalian IgG, like the antigen-binding fragment (Fab) with complementarity determining regions (CDR) and crystallizable fragments (Fc). However, the IgY antibody lacks a hinge region and has a long heavy chain (Lee *et al*., 2017). The comparison of IgG and IgY immunoglobulins are given in Table 1.

Chicken IgY production

Chicken IgY name was proposed by Leslie and Clem in 1969 and is different from IgG. IgY is the main low molecular weight immunoglobulin present in serum of chicken and egg yolk. According to the type of antigen, predominant immunocompetent cells and antibody class, the immune response in chicken can be classified as primary or secondary immune response (Gurjar *et al*., 2013). The primary immune response is characterized by the synthesis of IgM producing cells rather than IgY producing cells with an incipient immune memory. Interestingly, the secondary immune response has higher production of IgY which leads to a solid immune memory than IgG (Meunier *et al*., 2017; Ou *et al*., 2017). Transfer of IgY antibodies occurs by their translocation into the egg yolk (Merrill and Grindstaff, 2014; Bernardini *et al*., 2017). However, the pathogen neutralization ability of IgY in the immunological platform is predominantly influenced by the target antigen. The amount of IgY produced from chicken is influenced by the chicken breed, route, schedule of immunization, extraction and purification methods (Kovacs-Nolan and Mine, 2012).

The concentration of IgY in serum and egg yolk is around 5-20 mg/ml. The molecular mass [kDa] of chicken IgY is about ~ 180 kDa (light chain ~ 25 kDa each; heavy chain ~ 65-68 kDa). The Fc fragment is the most hydrophobic moiety present in IgY molecule (Schade *et al*., 2005). The production of antigen or fragment specific antibodies using chicken has ultimately gained its importance through high specific antibody retrieval at a low cost (Kovacs-Nolan and Mine, 2012; Diraviyam *et al*., 2014; Abbas *et al*., 2019; Pereira *et al*., 2019). The extinction coefficient (*i.e*. absorbance of a 10 mg/mL solution at 280 nm or optical density of a 1.0 mg/mL solution at 280 nm) for chicken is

Table 1: Comparison of Immunoglobulin G (IgG) and IgY.

Immunoglobulin	IgG	IgY	Reference
Species	Mammals	Birds and reptiles	Pereira *et al*., 2019
Source	Serum	Serum/ Egg yolk	Pereira *et al*., 2019
Molecular weight (kD)	150	180	Kovacs-Nolan and Mine 2004; Pereira *et al*., 2019
Isoelectric point (pI)	1.40	1.36	Li *et al*., 2002; Davalos-Pantoja *et al*., 2000
No. of constant domains	4	3	Kovacs-Nolan and Mine, 2004
Carbohydrate content (%)	2-4	4	Ohta *et al*., 1991
Hinge region	Yes	No	Lee *et al*., 2017; Pereira *et al*., 2019
Antigen valency	2	2	Zhang *et al*., 2017
Concentration (mg/ml)	10-12	Serum: 8-10	Wang *et al*., 2000
		Yolk: 10-20	Carlander, 2002
Percentage of total Ig	80	75	Kovacs-Nolan and Mine, 2004
pH stability	2-11	3.5-11	Pereira *et al*., 2019
Stable at >65°C	Yes	No	Kovacs-Nolan and Mine, 2004
Major serum antibody	Yes	Yes	Pereira *et al*., 2019
Mammalian complement binding	Yes	No	Kovacs-Nolan and Mine, 2004; Pereira *et al*., 2019
Rheumatoid factor binding	Yes	No	Pereira *et al*., 2019
Fc receptor binding	Yes	No	Pereira *et al*., 2019
Binding to protein A	Yes	No	Pereira *et al*., 2019
Binding to protein G	Yes	No	Pereira *et al*., 2019
Binding to protein L	Yes	No	Nilsson and Larsson, 2005

1.36 (Leslie and Clem, 1969). The IgY antibodies are mostly limited to antigen-specific (2-10%) with a productivity of 20 -40 grams per year from single chicken (Pauly *et al*., 2009; Abbas *et al*., 2019; Pereira *et al*., 2019). Approximately, 12 eggs contain 1 g of total IgY antibodies, which is equivalent to the total amount of IgG antibodies present in 100 mL of serum. Around 2.5 g of total IgY antibodies can be produced per chicken per month. In chicken targeted antigen-specific proteins or molecules administered through breast muscle of chicken (i.m) with various intervals and passive immunization is about to start from 4 weeks to a year since the last vaccination (Pereira *et al*., 2019). Unlike in mammal's polyclonal antibody production, the chicken IgY preparation is a non-invasive technique and the amount of immunoglobulin is estimated to be equal to that of 40 rabbits (Kovacs-Nolan and Mine, 2004).

The egg yolk is concentrated daily into the chicken ovarian follicle by the translocation of compounds from blood molecules (Fig 1). Among these compounds, IgY antibodies are most commonly deposited or concentrated in the follicles. The deposition of IgY in egg yolk usually follows a circadian rhythm pattern with five-day intervals between the passage of higher and lower concentrations of IgY (He *et al*., 2014). The IgY antibodies can be easily extracted from the egg yolk and the process does not require exhaustive procedures like bleeding of the hens.

IgY and its advantages

Passive immunization is generally considered to be only for a short period of effective prevention of infectious diseases similar to maternal colostrum. The existing polyclonal antibodies are generally produced using mice, rats, rabbits, sheep, goats and horses. The principle method of collection is limited through sera after the target antigen immunization. On a large scale and for industrial purposes, the existing polyclonal antibody production using mammals is less successful due to increased blood necessity in the view of producing significant antibodies for wider application (Kovacs-Nolan and Mine, 2004). In such circumstances, chicken IgY is an effective and alternative method with less animal usage and specific immunoglobulin production (Schade *et al*., 2005; Diraviyam *et al*., 2014; Santos *et al*., 2014; Abbas *et al*., 2019; Pereira *et al*., 2019).

In the present era, domestic animals have the capability for producing large scale therapeutics and other biological in infectious disease control. The utility of IgY from the eggs may overcome unnecessary animal usages and invasive techniques (Schade *et al*., 2005; Diraviyam *et al*., 2014; Santos *et al*., 2014; Pereira *et al*., 2019).

Additionally, IgY does not bind to mammals Fc receptor, rheumatoid factor, or proteins of complement (C1q and C3). Collectively, these features are the most important to prevent the occurrence of false-positive findings in diagnostic research platforms and make IgY a suitable innovation like an immune reagent (Santos *et al*., 2014; Lee *et al*., 2017). Thus enhance the need of IgY in various therapeutic areas where an anaphylactic component is unavoidable.

The production of monoclonal antibodies, such as single-chain fragment variable (scFv) by cloning the fragment antigen-binding (Fab) coding genes from the chicken B cell can be achieved easily (Junior *et al*., 2012; Nie *et al*., 2014; da Rocha *et al*., 2017; Borges *et al*., 2018). Similarly another interesting fact in IgY technology is, it can be used for a long time under various storage conditions without disturbing its functionality such as for a month at 37°C; six months at room temperature and 5-10 years at

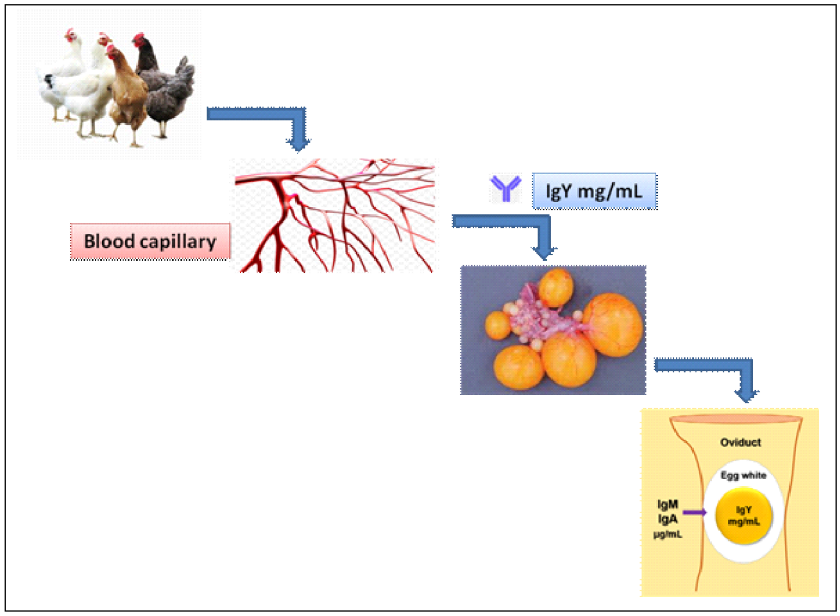

Fig 1: IgY antibody translocation from chicken blood to egg yolk in ovarian follicle and IgM, IgA deposition into egg whites through oviduct epithelium.

4°C (Larsson *et al*., 1993; Nilsson *et al*., 2012; Abbas *et al*., 2019). Due to less expensive, more productivity, non-invasive, no cross-reactivity, heat tolerance and pH stability the IgY can be used as a new modality for various clinical purposes (Schade *et al*., 2005; Kovacs-Nolan and Mine, 2012; Diraviyam *et al*., 2014; Abbas *et al*., 2019; Pereira *et al*., 2019).

IgY applications in human and animal practices

The chicken immunoglobulin (IgY) is widely studied in immunology and medical interventions. The major IgY applications in medical practices are antibacterial, antiviral, antifungal, antiallergic, immune modulator, growth promoter, antivenom, prophylaxis of poisoning, antiobesity, nutritive supplements and diagnostics (Schade *et al*., 2005; Kovacs-Nolan and Mine, 2012; Diraviyam *et al*., 2014; Pereira *et al*., 2019). The detailed information on immunoprophylactic and immunotherapeutic role of IgY in human and animals practice has been furnished with appropriate literature survey for easy understanding.

Immunoprophylactic role of IgY in human and animal diseases

The passive immunization of IgY may produce significant prevention against various infectious diseases and snake poisoning. However, in comparison with active immunization techniques, passive immunization produces a strong and short span of immunity. In a catastrophic pandemic situation, IgY could be used due to the easy and quick production ability with impressive results. The passive prophylactic role of IgY in human and animal species has been studied under various conditions. In humans rotaviral diarrohea (Rahman *et al*., 2012), pseudomonas infections (Nilsson *et al*., 2008), candidiosis (Wilhelmson *et al*., 2005), dengue (Fink *et al*., 2017) and streptococcosis (Zhou *et al*., 2003) were studied. In animals, salmonellosis (Yokoyama *et al*., 1998; Gurtler *et al*., 2004), colibacillosis (Ikemori *et al*., 1992; Yokoyama *et al*., 1992; Li *et al*., 2009; Mahdavi *et al*., 2010; Germine *et al*., 2011), rotaviral diarrhea (Vega *et al*., 2011), infectious bursal disease (Yousif *et al*., 2006), porcine epidemic diarrhea (Kweon *et al*., 2000), coccidiosis (Lee *et al*., 2009a,b), clostridial infections (Pizarro-Guajardo *et al*., 2017), vibriosis (Hirai *et al*., 2010), staphylococcosis (Leclaire *et al*., 2002), streptococcosis (Zhou *et al*., 2003), white spot syndrome (Lu *et al*., 2009), flu (Tsukamoto *et al*., 2011), hantaviral pneumonia (Haese *et al*., 2015), tetanus (Selim *et al*., 2015), snake poisoning (Paul *et al*., 2007; Meenatchisundaram *et al*., 2008a,b; de Almeida *et al*., 2008; Lee *et al*., 2016), rabies (Motoi *et al*., 2005) and phytate phosphate toxicity (Bobeck *et al*., 2016) were studied.

Immunotherapeutic role of IgY in human and animal diseases

The IgY derived against specific pathogen may be helpful in the treatment of various infectious diseases with high neutralization ability resulting in increased recovery rate and reduced host damage through elimination of microbial load. The short lifespan of IgY in mammalian host (maximum 36 hours) results in increased clearance leading to easy elimination of infectious agents (da Silva and Tambourgi, 2010). Immunotherapeutic role of IgY were studied extensively in humans against common infectious diseases like rotaviral diseases (Sarker *et al.,* 2001; Rahman *et al.,* 2012) and streptococcosis (Zhou *et al*., 2003). Similarly in animals, IgY has been used as immunotherapeutic in various fungal, bacterial, parasitic and viral diseases *viz*., colibacillosis (Cook *et al.,* 2005; Germine *et al*., 2011), salmonellosis (Gurtler *et al.,* 2004), canine parvoviral enteritis (Van Nguyen *et al*., 2006; Suartini *et al*., 2014; Naveenkumar *et al*., 2019) and trypanasomiasis (Sampaio *et al*., 2014). Moreover, it has also been used in treatment of snake bite cases (Paul *et al*., 2007; Meenatchisundaram *et al*., 2008a,b; de Almeida *et al*., 2008; Lee *et al*., 2016).

Immunodiagnostic role of IgY in human and animal diseases

As of now, mammalian infectious diseases are diagnosed with antigen and or antibody detection. In large scale surveillance and other practices, antibody detection with a primary binding assay such as enzymatic linked immunosorbent assay (ELISA), lateral fluorescent assays, immune chromatography, *etc*. are adopted The major drawback of current diagnostic method is cross-reactivity with other similar pathogens which reduce the specificity (Parida *et al*., 2008; Magtoto *et al*., 2019). In those areas, IgY based ELISA will be an outstanding diagnostic kit as it avoid false-positive results. Due to IgY specific targeted pathogen in ELISA, cross-reactivity with other similar pathogens would not happen (Reddy *et al*., 2013; Shapouri *et al*., 2018; Constantin *et al*., 2020). Additionally, IgY technique will be used for immunodiagnostics such as antigen-binding repertoire, which is achieved by gene conversion using the insertion of segments from pseudogenes (Kaiser, 2012); avidity maturation; enzyme and fluorescence antibody conjugation and immune-gold beads antibody labeling (Shapouri *et al*., 2018; Constantin *et al*., 2020).

Role of IgY in biowar

Immunoglobulin Y has a significant application in the protection against bioterrorism microbes *viz*., *Staphylococcus* enterotoxin B (Leclaire *et al*., 2002). In biowar situations, the production of vaccines is a much challenging task for large scale population. In such circumstances, passive immunization against the bio-warfare agents will be much beneficial for large population, though the prevention is not for a long phase (Pereira *et al*., 2019). Microbial resistance can also be counteracted by the use of IgY technology (Diraviyam *et al*., 2014). Dissemination of field level therapeutic as well as passive prevention methods is much affordable by using IgY.

Role of IgY in food preservation and other scopes

In food industry chemical preservatives are commonly used to avoid microbial spoilage. Biological preservatives are

required by the industries for better stability. In such circumstances IgY has been found to be very good as natural preservative. Due to the specificity in neutralization of spoilage organisms and merit of quick clearance in mammalians gastrointestinal tract, IgY is preferred in food industries (Baloch *et al*., 2015; Zorriehzahra *et al*., 2016). The use of earlier IgY against common spoilage organisms *viz*., *Listeria monocytogenes, Shewanella putrefaciens* and *Pseudomonas fluorescens* (Sui *et al*., 2011; Xu *et al*., 2012; Zhang *et al*., 2015) has been reported.

Additionally, IgY utility were reported in various modes such as anti-obesity (Hirose *et al*., 2013; Tarigan *et al*., 2016), anti-allergy with immunomodulatory effects (Wei-Xu *et al*., 2016) and as growth promoter (Cook, 2002; 2004). Potential zoonotic agent vaccines can also be developed which will reduce the chances of zoonosis. *Brucella* vaccine strain with IgY has been studied *in-vitro* with good success rate (Moreno *et al*., 2016). Zoonotic pathogen intervention tools with reducing health professional risk are much warranted theme in the current scenario (Moreno *et al*., 2016; Hans *et al*., 2020).

The current scenario with the pandemic load of COVID-19, possess the need for suitable antiviral vaccine for devising control strategies. The challenging attributes of COVID-19 virus have resulted in the delay in the production of vaccines and other suitable medical counter measures (Wu *et al*., 2020). In such circumstances, IgY is a wonderful research area in which the production time will be minimal compared with vaccine production (Constantin *et al*., 2020). The role of antigen site determination is a challenging task for the current pandemic (Wu *et al*., 2020). Interestingly the role of IgY in SARS-CoV was studied in *in-vitro* set up and found to be more effective in the prevention and control of the viral load (Fu *et al*., 2006). Though the similarities between SARS-CoV and COVID-19 (SARS-CoV-2) were well documented in terms of phylogenicity and respiratory system effect, still it needs to be studied effectively both in-*vitro* and *in vivo* for IgY based therapeutic approach (Wu *et al*., 2020). In emerging, re-emerging infections and other unpredictable situations IgY could be a suitable candidate to control the spectrum of the epidemic in a short period (Constantin *et al*., 2020).

CONCLUSION/ FUTURE PERSPECTIVES

Active immunization through a potent vaccine strain is very important to control epidemics of infectious diseases. Due to pathogen mutation, the epidemics are unpredictable resulting in delays in finding active immunization. In those circumstances, passive immunization with chicken antibodies (IgY) is a recent techniques with various positive factors *viz*., low cost, non-invasive, reduced animal numbers, non-allergic, non-cross-reactive and increased production. In the present scenario in finding alternate way for antimicrobials, IgY is one of the promising techniques in therapeutics. Additionally, antimicrobial resistance issues will be manageable while using IgY. The specificity of IgY is a fully dependable factor on targeted antigen used for vaccination. In a nutshell, IgY is a sound technique in the immunology of human and animal clinical practice and the role of IgY in all areas of clinical practice must be studied in future.

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Conflict of interest

The authors report no conflict of interest.

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