*Journal of Applied Veterinary Sciences, 6(2): 67 - 91 (.April, 2021)*
**ISSN: Online: 2090-3308, Print: 1687-4072**
**Journal homepage : https://javs.journals.ekb.eg**

# Chicken Egg Yolk-IgY: Passive Immunization Promising Targeted Therapy of COVID-19 Pandemic

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## ABSTRACT

The world is threatened by the lethal effects of *Coronaviruses* (CoVs) that associated with major respiratory disorders. The CoVs are large, enveloped, positive-stranded RNA and therefore, it is considered a complicated virus structure. The spike (S) is the major protein that exists in CoV's structure regulates the viral access to the host using *angiotensin-converting enzyme 2* (ACE2) receptor for both severe acute respiratory syndrome *Coronaviruses*-2 (SARS-CoV-2) and/or SARS-CoV initiates the animal/human disease and elevated immune responses. The receptor binding domain (RBD) in the S-proteins is essential for the CoVs-gene sequences; however the literature discussed CoV S-protein in relation to receptor-binding is little. Therefore, finding a natural antiviral to limit the spreading of CoVs is important. Herein, Immunoglobulin (Ig) Y is considered a kind of passive immunizations, represents 60% of the egg-yolk and can preventing many infectious diseases due to the particularities of IgY-molecule shape functional bioactivity to combat the pathogens. Comparing IgY to other Ig(s) antibodies, it makes the minimal animal stress, produces the lowest responses to mammalian factors. Besides, IgY is the most cost-effective extraction using the precipitation which is efficient protocol produce about (95% purity) of antibodies. We reported that IgY has a killing power to bacterial infections in broilers which beneficial to produce hygienic meat. Also, IgY-technology has a global application for treatment of viral infections and cancers. Interestingly, the anti-SARS CoV-2 single-chain fragments variable (scFv) IgY-antibodies can separate the virus from the host cell ACE2-and RBD-receptors. The expressed scFv-antibody can be assigned to be a specific-antigen binding for the potential neutralization which make a better understanding of the host-cycle range of SARS-CoV2 to adapt the ACE2 for the SARS-CoV-2 infection. Therefore, we concluded in this review that the IgY is promising to be a therapeutic target of a novel *Coronaviruses* disease-19 (COVID-19) pandemic.

***Review Article:***
DOI:https://dx.doi.org/10.21608/javs.2021.164324

***Received :11 March, 2021.***
***Accepted :15 April,2021.***
***Published in April, 2021.***



**Keywords**: COVID-19, Egg-yolk, Immunoglobulin Y, Passive immunity, Therapy.

***J. Appl. Vet. Sci., 6(2): 67 – 91.***

## INTRODUCTION

Humoral immunization refers to administration of natural antibodies (Igs) to combat the infections **(Casadevall and Scharff, 1995)**. It might be initiated by natural or artificial mechanism **(Baxter, 2007)**. Such as antibodies produced by the mother and then transferred to the offspring **(Rehan *et al.*, 2019)**. In mammals passive immunity is originated from placenta to feed the embryo; particularly during the last tri-master of pregnancy. Besides, it might be given by colostrum which contains antibodies **(Helmenstine, 2020)**.

While, in birds, this kind of immunity is also occurred in similar way by transfer the immunoglobulin Y (IgY) to egg-yolk to protect the growth of the embryo **(Patterson *et al.*, 1962; Rose *et al.*, 1974; Hussein *et al.*, 2020; Rehan *et al.*, 2020)**.

And, this IgY is circulating in blood of chicks for the first two weeks after hatching **(Hamal *et al.*, 2006; Rehan *et al.*, 2019; Rehan *et al.*, 2020)**.

On the other hand, this kind of immunity can be induced artificially by transferring antibodies by different routes of administrations. Therefore, to induce this particular-immunity we should produce then introduce similar protective antibodies against a desire diseased **(Chalghoumi *et al.*, 2009)**. Previously, it was reported that *intra*-nasal administration of IgYs achieved a great success for combating viral infections in animals (e.g., *influenza*-, *Sendai*-, and *respiratory syncytial-virus*), as well as in human (e.g., *influenza-A*-, *influenza-B*-, *Coxsackie*-, and *rhino-viruses*) **(Weltzin and Monath, 1999)**. The IgY molecule has a similar structure to that of IgG, with two heavy chain (67 to 70kDa), and two light chain with 25 kDa. The light chains have one constant region and one variable region, similar to IgG. The difference is that IgG has three constant regions at the heavy chains but the IgY has the four constant regions **(Schade *et al.*, 2005)**. Also, *Rotavirus*-caused acute diarrhea **(Rahman *et al.*, 2012)**.

Besides, IgY is a therapeutic targeted for various viral infections, including *Coronaviruses* (COVs) which recently threat the populations' life. The CoV has a complicated RNA-structure and rich of proteins **(Enjuanes *et al.*, 2006; Perlman and Netland, 2009; Kumar *et al.*, 2020)**. The most important protein in the CoV is the spike (S) protein that activates virus and regulates its access to host **(Li, 2016)**. Importantly, the *angiotensin-converting enzyme 2* (ACE2) is the master receptor for SARS-CoV-2 or SARS-CoV, that initiates the host (human's/animal's) immune responses **(Watanabe *et al.*, 2019)**. Consequently, it activates the receptor binding domain (RBD) in the S-proteins to change the CoVs-gene phenotype **(Watanabe *et al.*, 2019; Wahba *et al.*, 2020; Zhou *et al.*, 2020)**.

Chicken-IgY has a unique pathway to the CoV's infection because the anti-SARS CoV-2 single-chain fragments variable (scFv) IgY-antibodies can prevent the attachment of the virus to ACE2-receptors of host cell and to RBDs **(Finlay *et al.*, 2005; Hof *et al.*, 2008; Iwamoto *et al.*, 2009; Pansri *et al.*,, 2009; Green *et al.*, 2015; Neher *et al.*, 2020)**. The expressed scFv-antibody could be beneficial to the specific-antigen binding for the potential neutralization **(Schmitz *et al.*, 2000)**. Therefore, the aim of this review to highlight the possibility of IgY to combat the *Coronavirus* disease-19 (COVID-19) pandemic.

## "Coronavirus (COVs) And Its Structure"

CoVs are related to the *Coronaviridae* family in the order *Nidovirales* **(Enjuanes *et al.*, 2006; Perlman and Netland, 2009)**. These viruses are categorized into four genera: *α-CoVs, β-CoVs, γ-CoVs*, and *δ-CoVs*. Moreover, the *α*-and, *β-CoVs* infect mammals; *γ-CoVs* infect chickens, and *δ-CoVs* infect both mammalian and chickens. The *α-CoVs* included for instance, human CoVs NL63, porcine transmissible gastroenteritis *Coronavirus*, porcine epidemic diarrhoea, and porcine respiratory CoVs. While, *β-CoVs* included sever acute respiratory syndrome-*Coronaviruses* (SARS-CoV), Middle East respiratory syndrome *Coronaviruses* (MERS-CoV), bat CoVs, mouse hepatitis CoV, bovine CoV, and human CoV OC43. The *γ-and δ-CoVs* included chicken infectious bronchitis CoV, and porcine *δ-CoV*. Importantly, all CoVs (Fig.1) are elongated, enveloped, and have positive-stranded RNA. Uniquely, they have a master genome compared to the other RNA-viruses, ranged (27~32 kb). The genome is packed inside a helical capsid, formed via the nucleocapsid (N) protein and then encapsulated in an envelope.

There are three proteins have big roles in the assistance of CoVs' activity: i) membrane (M) protein, ii) the envelope (E) protein which incorporated into virus assembly, and iii) the spike (S) protein which regulates the viral access to host. Some CoVs encoded an envelope-linked hemagglutinin-esterase (HE) protein. The S-protein makes great entrance onto the virus surface, giving the crown shape of CoVs from its superficial layer. That is the reason why the Latin name of *Corona* means crown. Therefore, the S-protein has an essential role to regulate CoV's entry to the host, tissue tropism and then activating the host's immune responses **(Li, 2016)**. The 2019 novel *Coronavirus* (2019-nCoV) is related to RNA-recombination of CoVs **(Kumar *et al.*, 2019)**. This SARS-CoVs causing the global pandemic has been revealed in fresh detail of a bid to learn more about its structure targeted to develop a specific-vaccine of the COVID-19. Therefore, the structure of CoVs provides a basis of the design of new antiviral-drugs.

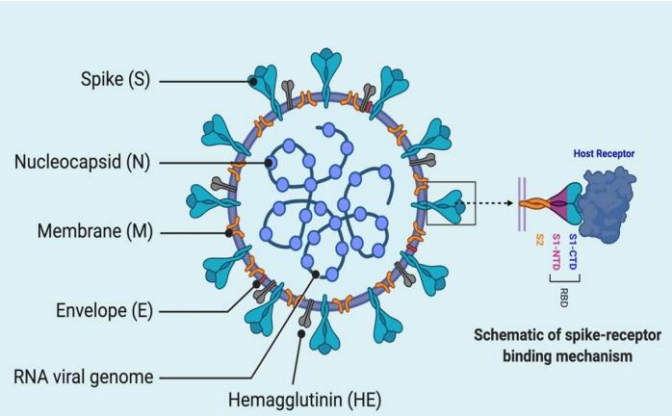


Fig. 1. Coronavirus structure and spike protein binding to host cell receptor (the figure was made using biorender.com).

**CoV spike (S) protein is the master key for binding the virus to the host immune cells**

Focusing on studying the CoV S-protein is the key of preventing infection. However; other mysteries about the CoVs's behavior remain unclear. A better understanding of what happens when a virus first encounters a human cell as it begins infection is demanded in detail **(Andersen *et al.*, 2020; Gao *et al.*, 2020)**. Unfortunately, the literature discussing CoV S-protein in relation to receptor-binding, membrane-fusion through conformational alterations, internalization, tissue tropism, and targets for vaccine discovery is little **(Kumar *et al.*, 2019)**. Therefore, to create a vaccine or therapy should think about the CoVs transmembrane spike (S) glycoprotein which is consist the superficial layer of virus which is the responsible for its access to the host cells. The *angiotensin-converting enzyme 2* (ACE2) receptor of the S-glycoprotein is essential for the access of virus to the host cell **(Watanab *et al.*, 2019)**. Its clearly now that the SARS-CoV-2 infection resulted from the binding to ACE2-receptors in mammal cells. Therefore, the shifting of ACE2 levels/expressions may open a new gate for the potential host of SARS-CoV-2 **(Sun *et al.*, 2020)**. Also, the RBD in the S-protein has an important role in adjusting the CoVs gene-sequences **(Wahba *et al.*, 2020; Zhou *et al.*, 2020)**. The researchers found that there are six RBD-amino acids bound to ACE2-receptors and had the ability to identify the host range of SARS-CoVs **(Wan *et al.*, 2020)**. These six domains of SARS-CoV were as the following; Y442, L472, N479, D480, T487, and Y4911, related to five important amino acids, L455, F486, Q493, S494, N501, and Y505, respectively in SARS-CoV-27 on the RBD from S-proteins responsible of contact between virus and host. Mostly of these six domains are not identical by comparing SARS-CoV-2 to SARS-CoV. Based on the molecular studies **(Walls *et al.*, 2020; Wan *et al.*, 2020; Wrapp *et al.*, 2020)** and biochemical researches **(Letko *et al.*, 2020; Wrapp *et al.*, 2020; Zhou *et al.*, 2020**), SARS-CoV-2 had a RBD which attaches strongly to ACE2-receptors in humans, ferrets, and cats with high-receptor homology **(Wan *et al.*, 2020)**. Furthermore, SARS-CoV-2 is probably attached to human-ACE2 with high-affinity. The computational analyses suspected that the cross-reaction is not perfectly detected **(Wan *et al.*, 2020)**, and therefore the RBD-sequencing is not identical to the SARS-CoV to the optimum receptor-binding **(Sheahan *et al.*, 2008; Wan *et al.*, 2020)**. It is noticeable that the high-affinity binding of the SARS-CoV-2 S-protein to ACE2-receptors was identical to a natural selection of a human-like ACE2 which allows another optimal-binding solution to generate. It is revealed that SARS-CoV-2 was not the target for the potential manipulations **(Andersen *et al.*, 2020)**. Meanwhile, the genomics confirmed that SARS-CoV-2 is originated from the bat SARS/SARS-like CoVs; whereas bats considered as a natural reservoir. Further, SARS-CoV-2 made specific-mutations through critical process of non-detected genomic regions at the CoV's structure (illustrated in Fig. (1) in order to generate a novel infectious CoVs. This mutation of SARS-CoV-2 has particular impacts on its pathway, which rapidly adapted to a new environment. Additionally, nine putative recombination patterns of SARS-CoV-2 are detected, which encompass S-glycoprotein, RdRp, helicase, and open-reading frame 3a (ORF3a). Six of these nine recombination regions are identified in the spike (S)-gene, and then had an essential role for the evolution. Also, the CoVs can be genetically modified to provide the immune-adaptations inside the animal's body and then accustomed to the human host. These combined natural pathways made the novelty of SARS-CoV-2 in human or animal hosts **(Rehman *et al.*, 2020)**. It is clear that SARS-CoV-2 involved in numerous clinical researches of SARS-CoV-like CoVs. Meanwhile, the RBD of SARS-CoV-2 is specifically selected for the binding to human-ACE2 receptors **(Sheahan *et al.*, 2008; Wan *et al.*, 2020)**. Furthermore, understanding the genomic-sequences might enable us to identify the multi-reverse genetic systems targeted for $\beta$-CoVs **(Cui *et al.*, 2019)**. In contrary, it is stated that SARS CoV-2 is not resulted from any earlier virus backbone **(Almazán *et al.*, 2014)**. Instead, two scenarios explained the natural pathways to SARS-CoV-2 and their hosts, first scenarios found in animal prior to zoonotic transfer; and second scenarios found in human after zoonotic transfer **(Andersen *et al.*, 2020)**. Therefore, it is found that ACE2 is the master receptor for both SARS-CoV-2 and/or SARS-CoV. The potentiality of host range of SARS-CoV-2 can be identified by the residual effects of ACE2, and the impacts of S-proteins on the viral activity. Abundantly, pets, pangolin and *Circetidae* mammals protected the most of master-residues for the binding-activity with S-protein from SARS-CoV or SARS-CoV-2. Thus, the formulation processes among pets/pangolin/Chinese hamster ACE2-receptors, and SARS-CoV/SARS-CoV-2 S-protein is enhanced by homology modeling. It is well-known that N82 in ACE2 evidenced a stronger relationship of SARS-CoV-2 S-protein compared to M82. All these information would make a better understanding of the host range of SARS-CoV-2 and creating a novelty of the optimization of ACE2 for SARS-CoV-2 infection **(Luan *et al.*, 2020)**. Currently, the COVID-19 pandemic, caused by SARS-CoV-2, is the more dangerous disease compared to the previous outbreaks caused by other CoVs, such as SARS-CoV and MERS-CoV. So, human-ACE2 is confirmed to be the receptor for the SARS-CoV-2 S-protein. Hence, variations in the viral S-protein make the cross-species transmission of the virus, genetic mutations in the host

receptor of ACE2, and/or the sensitivity/resistance to the CoV's infection might be occurred; so the full understanding of the attaching of the proteins encoded by different human-ACE2 receptors with SARS-CoV-2 S-protein would be important. For instance; coding variants of ACE2 corresponding to the reported binding sites with CoV S-protein are identified, and the molecular models of these variants are induced by homology modeling. Then, the models are involved in the native ACE2-and ACE2-S-protein complex, to detect the structural alterations in the ACE2 variants, and consequently to detect the inter-molecular interactions with SARS-CoV-2 S-proteins **(Hussain *et al.*, 2020)**.

## "SARS-CoV-2 Replication And Pathogenesis"

The schematic cartoon in Fig. (2) shows the replication cycle of SARS-CoV-2 where it accessed to the host of the ACE2-receptor. For instance, human-ACE2 allocated to the lower respiratory tract, acted as the cell-receptor for SARS-CoV mediating the cross-species among human populations. Furthermore, the virion S-glycoprotein occurred on the outer surface of CoVs binds to the ACE2-receptor. Also, SARS-CoV-2 S-protein ACE2-binding efficiency is up to 20-fold higher than that of SARS-CoV. The cleavage of trimer S-protein is activated through the cell surface-linked-*transmembrane protease serine 2* (TMPRSS2) and *cathepsin* in SARS-CoV. This virus is characterized by club-like spikes on the surface, and a unique re[lication strategy. Cell entry of CoVs depend on binding of S-protein to cellular receptors and on S-protein priming be host cell *protease* (Fig. 2). Meanwhile, the predicted molecules incorporated into membrane invagination for endocytosis process of SARS-CoV-2 are still unknown. Meanwhile, the SARS-CoV-2 is transmitted among people and considered a pandemic disease other than that of SARS-CoV which causes severe infectious disease with high mortality rate. In addition, S-glycoprotein consisted of two subunits, S1 and S2 - It is well-known that S1 is powerful agent for the identification of the virus-host range and tissue-tropism through RBD, while S2 incorporated into the virus-cell membrane fusion through two tandem domains, heptad repeats 1 and heptad repeats 2.

The RNA of CoVs of almost 30,000 nucleotides encoded structural-and nonstructural-proteins (SP-and NSP) of the virus, had an essential role in viral RNA-synthesis (named *replicase-transcriptase proteins*). For instance, the CoV NSP-1 has been shown to promote cellular mRNA degradation, block host cell translation, and inhibit the innate immune response to virus infection. Also, deleting of the NSP-1 coding region in infectious clones prevented the virus from productivity infecting cultured cells. Thererfore, SP/NSP cocktail vaccine containing structural-and nonstructural-proteins (SP-and NSP) of the virus would enhance effective complementary immune responses. On the minimum cases, one niche-specific protein, NSP2, one structural-protein, and the N-proteins, are emerged into the RNA-synthesis of the CoVs. Moreover, the expression level of the CoV *replicase-transcriptase* protein genes is regulated by the transcription of the RNA-genomics. These proteins are encoded in ORF1a and ORF1b, and therefore initially synthesized as two large-polyproteins, pp1a and pp1ab. Furthermore, the synthesis of pp1ab emerged from frame-shifting of ribosomes in the translation process of ORF1a. These polyproteins are cleaved through virus-encoded *proteinases* with *papain*-like protein and *chymotrypsin*-like folds into sixteen proteins during or after the synthesis of pp1ab. Meanwhile, NSP1 to NSP11 is encoded in ORF1a, and NSP12 to NSP16 is encoded in ORF1b. The *replicase-transcriptase* proteins, along with cellular proteins of viruses, performed into membrane-bound *replication-transcription complexes* (RTC). These complexes are occurred at the perinuclear regions and linked with double-membrane vesicles. Hydrophobic transmembrane domains were occurred in NSP3, NSP4, and NSP6 in order to anchor the nascent pp1a/pp1ab-polyproteins to membranes in the initial step of RTC-process. Further, the virion-containing vesicles fuse with the plasma membrane of the cell in order to generate the viral activities. Then, the virus binds to a novel targeted-cell, and consequently the cycle is regularly repeated **(Sapkota, 2020)**.

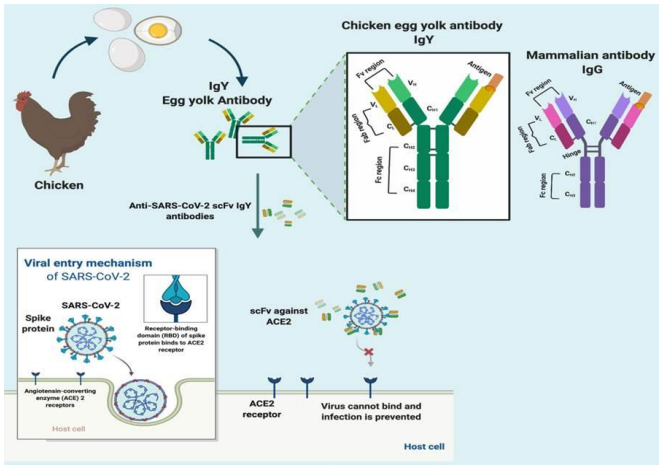


Fig. 2. SARS-Cov-2 Replication Cycle (The figure was made using Biorender.com).

## IGY-Antibodies

**IgY discovery:** it was found that the extracted yolks from the immunized hens'eggs with *tetanus toxin* potentially protected mice from the deadly toxins due to the neutralization antibodies in the eggs **(Chacana *et al.*, 2004)**. This discovery is totally overlooked until 1959, when Russell and Burch **(Balls, 2010)** made an established protocol discussing the significance of animal welfare and their ethics issues. After 10 years, it

is referred that the term "IgY" was commonly related to the antibodies of hens, collected from egg-yolk, because these antibodies were identified as not quite the same as their mammalian subjects (IgG) **(Leslie and Clem, 1969)**. Therefore, from 1996 until 2016, IgY-technology has generated globally acknowledged to assign the use of egg-yolk antibodies for multitherapeutics **(Schade *et al.*, 2005)**. Recently, on 2020, Egyptian team headed by Dr. Ibrahim F. Rehan, revealed that IgY could be used to improve the health and welfare of chickens **(Rehan *et al.*, 2020)**, and to limit drug/antibiotics used in broiler farms **(Hussein *et al.*, 2020)**.

**Benefits of IgY-technology:**

**O**ne of the most benefits of IgY-technology is that delivered from the eggs and not from the sera, which support animal welfare **(Gruber and Hartung, 2004)**. Other benefits of using IgY-applications are, i) these antibodies activate the host's immune system making the phylogenic-characters more valid in mammals and in avian as well **(Nilsson and Larsson, 2005)**; ii) nonrecognition of Fc-receptors in mammalian improves usage of IgY-technology **(Schade *et al.*, 2005)**; and iii) the inability of IgY to block the complement activation of mammalian *in vitro* and/or *in vivo* **(Carlander and Larsson, 2001; Sesarman *et al.*, 2008)**. Altogether, with the usual beneficial uses of eggs as passive immunization, might improve the IgY-technology; particularly one chicken produces her antibodies for two years. Additionally, egg yolk contains over 50 mg of antibodies, and nearly 5 mg is an antigen-specific **(Hatta *et al.*, 1993)**, which targeted to give novel gates of utilization for many biological demands.

Therefore, obtaining IgY from birds has multibenefits. For the biosafety, the IgY-extract does not induce further complications but definitely benefits the microbiota of the host. Besides, there is no cumulative effect of the IgY administrations on the meat muscles of animal and birds which is unlike a residual effects of antibiotics **(Li *et al.*, 2015)**. Interestingly, using IgY in a passive immunization is not restricted to the age, and therefore it can be used widely in individuals/patients suffered from pathophysiological syndromes, e.g., pregnant women and immunosuppressive patients. Also, IgY is a nontoxic agent in nature and it keeps the same efficacy power at 4˚C after lyophilizaton. The strategies of IgY-production costs are much lower when compared to the other strategies. In addition, IgY can be stored for several months and thus the transportation is easily performed compared to the other biological products **(Thu *et al.*, 2017)**. Moreover, the capsule of IgY can be kept stable inside the eggs until 48 weeks at 4˚C if not being extracted **(Jensenius *et al.*, 1981)**. Additionally, IgY can increase the binding-avidity for the antigens compared to mammalian-IgG **(Ikemori *et al.*, 1993)**. Also, IgY can be purified easily to combat conserved mammalian molecules than IgG-antibodies because of the phylogenic distance between mammals and birds **(Gassmann *et al.*, 1990)**. A lower antigen burden is demanded to produce the IgY for the activation of the immune responses **(Li *et al.*, 2015)**, and a large-scale production of the human consumption **(Sharma, 1999)**. As well, one chicken can produce 22 g of IgY yearly **(Pauly, 2011)**.

**Phylogenetic Structure of IgY Compared to Other Igs:**

The characteristic molecular structure of IgY is identical to that of mammalian one (shown in Fig. 3). Although each Igs incorporated the double-Fab-arms that involved each alternative domain of the light chain and the first two steady domains of the heavy one, their functional properties revealed a heavy chain additional domain found in IgY so it is consisted from 5 domains. However, IgG had triple-domains in the heavy chain; consisted of four heavy-chains of regular domains **(Thirumalai *et al.*, 2019)**. Also, the molecular weight of IgG was up to160 kDa. However, IgY was up to167 kDa (65 kDa "heavy chain" as well as 19 kDa "light chain") **(Sun *et al.*, 2001)**. In accordance to sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis, in a reducing condition, one band of up to 70 kDa and up to 25 kDa in heavy-and light-chain, respectively, for IgY was detected.

However, in non-reducing conditions, up to 180 kDa (a single band) is predicted **(Leiva *et al.*, 2019)**. Despite the hinge region of the IgY-molecule, it is less developed than the IgG-one. It does not mean that IgYs had poor flexibility **(Murphy, 2012)**, and therefore IgY-activity to bind antigens is generated **(Mudili *et al.*, 2015)**. However, binding to the Fc-region (such as IgM and IgE) is still unclear, due to the limitation of crystallization for the whole Fc-region **(Zhang *et al.*, 2017)**. This distinction in shape might also useful to reduce the capability of IgY to precipitate several antigens **(Warr *et al.*, 1995)**. Likewise, the IgG, the Fc-region of the IgY is concerned in numerous researches. It involved two carbohydrate aspect chains **(Chacana *et al.*, 2004)**. Moreover, IgY-molecule characterized by the isoelectric factor of 5.7~7.6; however IgG achieved the isoelectric character between 6.1~8.5. It is reported that the Fc-region of IgY is greater compared with IgG-one, and so the IgY is greater hydrophobically acted than IgG-one **(Schade *et al.*, 2005)**. As well, IgY is kept conditionally unchanged at 30 °C ~ 70 °C and at pH ≥ 11 **(Thu *et al.*, 2017)**.

Moreover, adding sugar particles makes IgY more stable to thermal inactivation (up to 40%) and after 8 hrs after digestion **(Chacana *et al.*, 2004)**, by *pancreatic enzymes* **(Hatta *et al.*, 1993)**. Moreover, adding of protein-rich solutions and/or nanocomposites

makes the IgY more resistant to acid and/or *proteolytic* inactivation **(Chacana *et al.*, 2004; Alustiza *et al.*, 2016)**. The *enzyme-linked immunosorbent assay* (ELISA) platform is most exploited immunoassay protocol used with IgY . Moreover, ELISA is the most accurate strategy to detect IgY in various infectious diseases. Although IgG is known as the weapon to detect pathogens, recently there is evidenced that IgY replaced IgG-molecule due to the IgY performed simply by a noninvasive strategy. For instance, it is delivered from hen's egg after being immunized with a recombinant protein, besides IgYs are heavily and economically produced than IgGs. The CoV S-protein provided an important role in the viral attachment, fusion, and entrance. By which, S-protein is targeted for drug discoveries and blockers. Here, RBD isrecognized in SARS-CoV-2 S-and RBD-protein are closely attached to human and ACE2-receptors. SARS-CoV-2 RBD achieved successively higher binding-affinity to ACE2-receptor than SARS-CoV RBD so inhibited the binding activities of SARS-CoV-2 RBD to the expression of ACE2 can block the virus entery. Therefore, altogether blocked the COV infection to host cells. SARS-CoV RBD-antibodies can interact with SARS-CoV-2 RBD-protein. Then, neutralize SARS-CoV-2, indicating a potential drug discovery to SARS-CoV-2/CoV infection **(Tai *et al.*, 2020)**.

## Mode of Action of Monoclonal Antibodies (MABS)- IgY

Several researches are currently ongoing in order to create drug discovery for treatment of SARS CoV-2 pandemic wishing to improve the global health of human as well as animal., These researches are little although the rapid transmission of SARS CoV-2 infection and the essential needs of vaccine development **(Bazan *et al.*, 2012)**. It is noticeable that the SARS CoV-2 is currently pandemic and uncontrolled, and it seemed likely to a *flu*-infection among humans affected for a long time. The mode of action of the chicken mAbs-IgY scFv (Fig. 3) was generated towards SARS CoV-2 S-protein through the passive immunization process. The scFv is commonly used as recombinant-antibody fragments, nearly 25 kDa in size, and consisted of two variable domains, of light and heavy chains, and associated with particular-linkers.

These domains can be induced toward the targeted-protein to generate the scFv-antibody, and then to identify the particular-antigens. Moreover, the smaller sized of the scFv-antibody had multibenefits in higher-yielding antibodies. These IgY-antibodies are resulted from the expressing in the host vectors such as *Escherichia coli*. Moreover, the antibody-affinity is aimed ultimately to have the antigen by numerous screening process followed by such mutagenesis of the complementarity-determining regions. Then, these numerous screening cycles activated the positive antibody scFv fragments. Moreover, the coupling of several processes *in vitro* will be essential to tailor the scFv monoclonal antibodies (mAbs)-IgY fragment, suggesting the particular-antigen. The production of mAbs incorporated in several fragment genes (VH/VL), resulted from PCR amplification from the B-cell will give a full-length scFv (**Figure 3**). These scFv-regions are cloned and then appeared in the host vectors (*E.coli*) for activating antigen-particular IgY, indicating the antibody-library **(Finlay *et al.*, 2005; Iwamoto *et al.*, 2009; Neher *et al.*, 2020)**. It is revealed that the antibody-library constructs are not changed through amplifications **(Hof *et al.*, 2008; Pansri *et al.*, 2009)**.

Recent researches confirmed that the production of IgY-mAbs applied according to the enzymatic processes targeting the scFv IgY-antibodies **(Green *et al.*, 2015)**. Interestingly, the chicken IgY-mAbs production towards SARS CoV-2 S-protein is ideal and fit for a large-scale production of antigen particular-antibodies (anti-SARS CoV-2 IgY-antibodies). This strategy blocks the antigen (SARS CoV-2) to bind ACE2-receptor occurred on the human-cell membrane, and therefore it blocked the access and multiplication of CoVs in the host cell. Furthermore, this IgY-technology offered minimal strategies variations compared to the other bacterial technologies **(Kato and Hanyu, 2013)** and also revealed that the expressed scFv-antibody objectives are markedly with maximum possibility of specific-antigen binding for the potential neutralizations **(Schmitz *et al.*, 2000)**. The immune derived antibody-libraries from chicken originated via phage display method can be smoothly identified. Moreover, the characteristic immunological advantages of chicken antibodies helped in development of particular-antibodies indicating a potential biomarker agent that permits the capture of characteristic Igs pertaining to their protein components **(Somasundaram *et al.*, 2020)**.

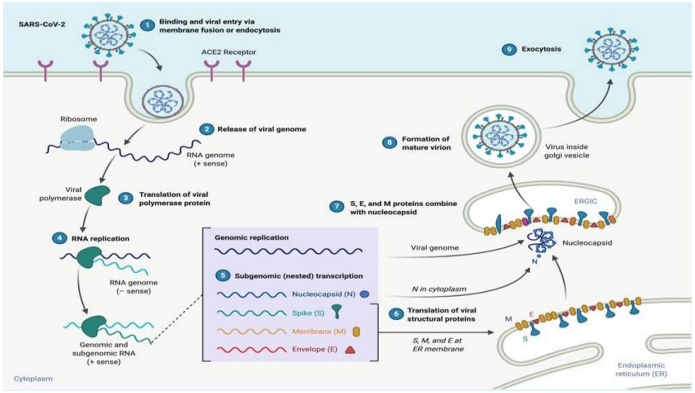


Fig. 3. Mechanism of action of anti-SARS CoV-2 scFv IgY-antibodies. This mechanism blocks the attachment of virus to the host cell receptors (ACE2). Structural comparison between both mammalian antibody (IgG) and chicken egg-yolk antibody (IgY). (the figure was made using biorender.com).

## Potential uses of monoclonal antibodies (mAbs)-IgY

Chicken mAbs-IgY using phage displayed a global strategy to treat multipathogenic infections; particularly the current pandemic SARS CoV-2 infection. Importantly, there is a demand to get detailed information about the mAbs-IgY. The mAbs-IgY-antibody towards SARS CoV-2 applied as a basic criteria, evaluated correctly the biomarker benefits of the disease **(Nishibori *et al.*, 2006; Daugherty, 2007)**. As well, numerous researches have been published to explain the role of chimeric scFv chicken IgY-antibodies in veterinary as well as in immunological applications **(Tsurushita *et al.*, 2004; Schusser *et al.*, 2013)**. Thermal and expression stability, aggregation capacity, solubility, and binding-efficacy revealed the strong correlation to the particular-scFv IgY-genes **(Wu *et al.*, 2012; Leighton *et al.*, 2015)**. The residuals alterations of scFv IgY-antibodies incorporated into the chicken scFv IgY after binding to chemicals such as chelating products and prodrugs targeted to immunotherapeutics **(Conroy *et al.*, 2014)**.

Altogether, is revealed the urgent demand of researches related to chicken mAbs-IgY towards particular antigens such as SARS CoV-2 and its therapy. Moreover, these receptors of antibody which targeted to the combined-particular sites are involved in potential therapeutics **(Yoon *et al.*, 2016)**. As well, chicken Igs fragments such as chFcR/L receptor molecules are detected exclusively as the first member of this chicken-antibody family, and have an essential role in regulating the immune system in mammals. Identifying these particular-pathways would be involved into novel approaches **(Sutton, 1989)**. The alterations of pathways in mammals should be identified in detail to create the functional role of these receptors on the evolution process of Igs **(Taylor *et al.*, 2009)**. The development of chicken mAbs-IgY using phage displayed strategy towards numerous pathogens have been described to find particular-scFv IgY-antibodies **(Taylor *et al.*, 2009)**. Additionally, the identifying of particular-scFv mAbs-IgY using phage displayed strategy in chickens was clearly effective, also the identifying mass production of the highly particular-mAbs-IgY scFv-antibodies revealed their immunological responses **(Taylor *et al.*, 2009)**.

On the other hand, these IgY-antibodies achieved a great inhibition toward snake-venoms which replaced the equine antibodies due to the minimum complications after administration of therapeutics **(Chen *et al.*, 2015)**. Recent researches stated that anti-Zika virus E-protein IgY-antibodies revealed the potentiality to be used in development of biomarker/vaccine which are found in the antibody-libraries, besides these scFv-antibody fragments presented a critical role to block the infections **(Choragi *et al.*, 2020; Mwale *et al.*, 2020; Somasundaram *et al.*, 2020)**.

## Isolation and purification of IgY:

Currently, the purified-IgY was made through a precipitation method. It can be done either by ammonium sulphate, polyethylene glycol (PEG) or column chromatographal strategies. To produce a highly purified-IgY (95%) and with an optimum immune responses, the sequential precipitation should be applied to 31% ammonium sulfate and 12% PEG **(Rajic *et al.*, 2009)**. Also, high‑column chromatography accomplished by NGC™ chromatography system can be used as a separation method of IgY. Therefore, this system provides more efficiency, accuracy, purity and functionality compared to the classical methods of column chromatography. Also, this multicolumn system automatically served multisteps for perfect purification in a one display run **(Elms, 2016)**. For instance, separation through binding-affinity of specific structures of original size and matrix to obtained the targeted product. It is reported that the separations of IgY-fragment can be obtained by the ion exchange column through diethylaminoethanol‑sepharose to detect both Fab-and Fc-fractions of high purity (88.7% and 90.1%, respectively). Therefore, the IgY-activity revealed its biological significance of several medical researches **(He *et al.*, 2016; Zhou *et al.*, 2020)**.

## Detection of IgY titer:

Detection of IgY titer can be analyzed by ELISA, as formerly reported **(Elms, 2016)**, **fraction collection and HPLC (Amro *et al.*, 2018)**. Microtiter plates have been used after optimizing the concentrations of mouse-IgG in carbonate–bicarbonate buffer at pH, 9.6, and kept over night at 4°C. Furthermore, the blocking is performed with 2% bovine serum albumin in peptone buffer saline (200 $\mu$L/well) at 37°C for 2 hrs. After triple-washing with phosphate-buffered saline and 0.1% Tween® detergent (0.05%), diluted IgY put per well and then kept at 37°C for 30 min. Another triple-washing is applied, the antibody of goat anti-chicken was once introduced and then kept at 37°C for 30 min. Accordingly, 3-, 30-, 5-, 50-tetramethylbenzidine (TMB, one hundred mL/well) used to be introduced once to one plates and incubated at 37°C for 5 min. This chemical reaction is stopped via including 2 M $H_2SO_4$ (50 mL/ well). Moreover, the absorbance values are detected at 450 nm, and also a microplate reader was used.

**IgY-Antibodies In Passive Immunization Therapy:**

**Impact of passive immunization on virus compared to bacteria**: multidrug resistance is a problem to correctly treat bacterial infections **(Pasteur *et al.*, 2010; Mogayzel *et al.*, 2013; Mogayzel *et al.*, 2014)**. However, just a collateral significance occurred in viral infections due to the substantial proportion of RTI are

typically viral infections in its acute form. Therefore, the treatment of these RTI infections depends on the antiviral drugs and gradually disappearing of clinical signs of viral disease. The viral pathogens influenced the viral proteins, and induced a selective ligation on the viral particle, suggesting an antiviral resistance **(McCaskill *et al.*, 2017; Ge and Sun, 2020)**. Therefore, the preventive therapy is the best choice to combat the viral infections; however there are limitations in the vaccine preparations. Also, we have to avoid many complications such as drug-drug-interactions, antigenic-variations, efficacy, and immune sensitivity. As far as threatening with a novel COVID-19 pandemic, no vaccine is developed to combat COV's infection and its multiplication **(Corti *et al.*, 2016)**, new products/strategy should be manufactured. Passive immunization is considered one of the novel strategies **(Sparrow *et al.*, 2017)**, which the polyclonal antibodies had multiorigins (sera of immunized animals/humans, and convalescing patients, etc.) **(Graham and Ambrosino, 2015; Shen *et al.*, 2020)**.

However, the polyclonal antibodies have some complications reflected on the patient's health and safety. In addition, it is costly expensive **(Ancuceanu and Neagu, 2016)**, besides the costs might significantly raise in respiratory infections due to the mutants of viral escaping which needs extra mAbs **(Casadevall *et al.*, 2004)**. In the immunological researches, the use of specific IgY was an interesting approach to the protect against viral infection. IgY is occurred in birds, reptiles, and amphibians with the same function of IgGs in mammals **(Alustiza *et al.*, 2016)**. Also, IgY is occurred in the serum; however its concentration is 100 times higher than the IgG-one. This make IgY promising used method as antiviral therapy.

**The multidisciplinary medical uses of IgY-antibodies**:

IgY-antibodies had been involved in therapeutic aids of bacteria **(Thomsen et al., 2016; Hussein et al., 2020; Rehan et al., 2020)** as well as of viruses **(Nguyen et al., 2010)**. It has characteristic properties mentioned as the following; tolerable because of the human's food-style, safely used in patients that have allergy to eating eggs hence the purified-IgY was mainly depleted of egg-albumin **(Rahman[a] et al., 2013)**. Moreover, the injected-IgY revealed its possibility to be used against viruses; for example IgY prevented rotavirus infection in pigs **(Vega et al., 2012)**. Moreover, systemic and topical-applications of IgY-molecule had revealed its ability to initiate the immune responses in mammals to involve the IgG-antibody. Altogether, it showed that IgY-antibody had antigenic effects; however this antibody cannot associate with Fc-receptors in mammals, thus minimal restrictions on this route are considered **(Torché et al., 2006)**. Hence, no allergic response to mice after administration of purified/non-purified-IgY, because its not generate an IgE's response **(Akita et al., 1999)**. In addition, IgY had no possibility to bind the Fc-receptors, so minimum inflammatory responses are concerned **(Kovacs‑Nolan and Mine, 2012)**. IgY tagged to either bacteria or virus, and facilitated the outlet of the pathogen from the hosts'gut in order to prevent bacterial/viral replication **(Xu et al., 2011)**. Passive immunization using IgY can be given to infected patients to develop rapid immune response. The immune-suppressed patients had characteristic benefits from this kind of passive immunization **(Muller et al., 2015; Zhang et al., 2017)**. Hence, IgY-molecule is beard with sial-terminal(s) **(Gilgunn et al., 2016)**, it had a long-term half‑life in vivo **(Liu, 2015)**, and therefore potentiated the anti‑pathogen responses to the infections **(Abbas et al., 2019)**.

## IgY & therapeutics:

The unique production of egg-yolk Igs attracted the scientists' attentions during the period 2010~2020, as evidenced by the growing number of literatures in this regard. Several research teams have evidenced that IgY was energetic in numerous pathogens/infections, as considerably reviewed elsewhere **(Rahman[a] *et al.*, 2013; Pereira *et al.*, 2019)**. As well, the patent activity is related to the therapeutic generated the application of IgY in human remedy. These patents generated since 2010 when numerous medical experiments linked to IgY-technology had been applied to human patients of different ages during the identical period. Besides, IgY had a bactericidal effect on gram-positive/negative bacteria **(Hussein *et al.*, 2020, Rehan *et al.*, 2020)**. Therefore, IgY could be used as targeted therapy for several infectious diseases concerning oral cavity, gastrointestinal tract, and metabolism.

## IgY can treat digestive tract infection:

Almost digestive tract infections such as gastritis, peptic ulcer, and gastric adenocarcinomas caused by the *H.pylori* **(Kodaman *et al.*, 2014)**. Currently, antibiotics are used to treat *H.pylori* infections that advance antimicrobial resistance. Consequently, IgY is an effective technology compared to other classical ways of treatments of pathogens. Oral-IgY can successfully diminish *urease* action; an essential enzyme for increasing multiplication of *H. pylori* **(Shimamoto *et al.*, 2002; Hong *et al.*, 2018; Mony *et al.*, 2019)**. It is reported that a patient suffered from *H.pylori*-related gastritis experienced treatments including IgY plus lansoprazole for about two months. The treatment confirmed synergistic impacts due to the IgY is fit for improving medication viability through relieving ulcer condition **(Suzuki *et al.*, 2004)**. Novel technique was described to generate IgY-therapy towards *H.pylori* after vaccinating hens with

recombinant antigens such as *urease* subunit *B, phospholipase A2* and adhesions **(Sheng *et al.*, 2014)**. Furthermore, it portrayed that IgY is related to antibody carrier (e.g., lecithin and cholesterol *nanoliposomes*).

In addition, recent procedure providing IgY-therapy against cytotoxin-delivering *H.pylori* strains, that was potential than non-cytotoxic strains. This study likewise portrayed where sixteen *H.pylori*-positive patients given one egg for ten days - It is a targeted therapy prompted a decrease of 90% infection susceptibility **(Wei *et al.*, 2017)**. Specifically, an elective patent utilization of IgY towards *H. pylori* is a preventive medicine is documented to utilize the therapy of *viral hepatitis*. It portrayed that titers of *Hepatitis B or Hepatitis C* are clearly decreased when participants given 1.92 to 2.88 g of IgY/day towards *H.pylori* **(Chen, 2012)**. It is the reason why the administration of IgY improved the cytotoxicity of killer-cells as well as IFN-γ, that helped the immune system to combat the viral infections. Additionally, a clinical research study was performed to test the efficiency of IgY on seventy-seven Asian patients (Vietnnam) suffered from *H.pylori* infections. Furthermore, a randomized, equal task, fourfold-blinded trial is started to assess the content of IgY towards *H.pylori* and *Lactobacillus*.

This study is planned to be finished by the end of 2020. Likewise, nutritional food mixed with IgY for *H.pylori*-related infection had likewise been created **(Horie *et al.*, 2004)**, where numerous of these items already marketed at Asia **(Thu *et al.*, 2017)**. One of the most widely recognized viruses is *norovirus* engaged with non-bacterial gastroenteritis **(Bányai *et al.*, 2018)**. A technique for enormous scope creation of explicit-IgY through inoculating hens is a recombinant *norovirus* **(Dai and Jiang, 2014)**. Also, they portrayed the inoculation of hens with P particles conveyed variable surface of antigens. As well, IgY is fit for hindering the official of *norovirus*-like particles to histo-blood group antigens which are important to get such contaminations **(Czakó *et al.*, 2012)**. Moreover, *norovirus*-specific-IgY stays constant either at 70°C for 30 min or pH up to 9 for 3 h **(Dai and Jiang, 2014)**. Numerous investigations *in vitro* likewise detailed predicting role of IgY for the prevention of *viral gastroenteritis* **(Dai *et al.*, 2012; Dai *et al.*, 2013)**. For instance, after performing a randomized placebo-controlled study it is confirmed that the adequacy of IgY towards *rotavirus* is a joint conducted for the preventive therapy **(Rahman *et al.*, 2012)**.

Fifty-two young patients of *rotavirus*-positive from Myanmar are recovered from IgY which provided by inoculating hens with two strains of *rotavirus*. The urgent demand for oral rehydration strategies and the duration of diarrhea and the fecal *rotavirus* shedding is diminished. Similarity, this finding agreed with the finding of those researchers that developed an explicit-IgY to combat principle *rotavirus* serotypes. They stated that IgY inhibited cell damage, as *rotavirus* adhesion, in a dose-dependent **(Rahman[b] *et al.*, 2013)**. On the other hand, a medical research completed in Myanmar with a newborn child milk containing particular-IgY suggested that the degree of secondary bacterial infection with *E.coli* diminished in pediatric patients **(Thu *et al.*, 2017)**. In this manner, oral administration of IgY towards *rotavirus* is probably permitted new baby patients to viably clear other pathogenic bacteria. Also, a randomized, placebo-treatment controlled study including one hundred and fifty babies experienced *rotavirus*-related *enteritis* is completed to interfere IgY-treatment towards *rotavirus* and a probiotic prophylaxis **(Xie *et al.*, 2013)**.

The consequences of the latest experiment demonstrated that IgY is efficient than probiotics for rapid recovery. IgY significantly declined the possibility of recurrence of diarrheic stools as well as viral shedding **(Xie *et al.*, 2013)**. IgY is used for the treatment of diarrhea caused by numerous pathogens **(Starzl, 2012)**. This item depended on IgY as the dynamic pharmaceutical ingredients plus cow-like colostrum such like the carrier matrix. To confirm this hypothesis, *in vivo*-study is done on three hundred youngsters experiencing acute diarrhea after being administrated seven gm of dried whole egg/child in addition to colostrum dissolved in 30 mL saline once daily up to three consequent days. The latest researches achieved a decrease in the bouts of diarrhea in those youngsters; their stool included one and/or more of the identified microbes-related **(Gaensbauer *et al.*, 2017)**. Meanwhile, a clinical trial (patent number: 2009-011446) utilizing IgY-specific antibodies is embraced in order to eradicate feaces of extended-spectrum *β-lactamase*-delivering *Klebsiella pneumoniae* and *E.coli* **(Jonsson *et al.*, 2015)**; however, this work is hold because of a high dropout rate.

## IgY can overcome dysbiosis:

Dysbiosis is the instability in the *microbiota* of intestine, and described by the occurrence of microbial species, that may cause the intestinal illnesses **(Honda and Littman, 2016)**. Many researches discussed the alteration of the functional properties of the intestinal microbiota after the oral administration of specific-IgY. This intestinal microbiota initiated numerous pathogens and colonized the intestinal tract **(Goepp, 2018)**. On the other hand, *in vivo* study described the influences of this particular-IgY on the intestinal-microbiome. To decide the recommended dose of IgY, for 3 months duration, a clinical trial treated one hundred patients, who are given capsules (1~4.5 g of specific-IgY).

Furthermore, the alterations of IgYs caused by inflammations are surveyed and the control of the gut microbiome was dissected through 16S RNA-sequence of fecal examples. Despite that IgY is a new trend of drug to develop the efficiency of the gut by providing beneficial microbes, caused by pathogenic microorganisms **(Hussein *et al.*, 2020; Rehan *et al.*, 2020)**, further examinations should study the adequacy of this application of relationship to different items as feed additives like probiotics.

**IgY can overcome toxins:**

The effective utilization of particular-IgY towards toxins had been examined. For example, An IgY-antibody used for neutralizing *Shiga* toxins **(Mitteness and Phillips, 2013)**, also the *lipopolysaccharide* (LPS) of *E.coli* **(Mitteness and Phillips, 2013; Rehan *et al.*, 2020)**. Also, in a murine model where a splash dried entire anti-toxin egg powder is orally administrated and therefore achieved an abundant decrease of mortality because of *Clostridium difficile* toxins. In mice, IgY used against *Shiga* toxin can neutralize the toxins and decreased the mortality in mice **(Neri *et al.*, 2011; Parma *et al.*, 2011)**. Additionally, it is stated that IgY purified from chickens vaccinated with a recombinant *C*-terminal heavy chain of *Clostridium botulinum* had the opportunity to combat *botulism* neurotoxins in mice **(You *et al.*, 2014)**.

On the other hand, it is exhibited that IgY increased towards *Clostridium perfringens* toxins and is fit for combating and killing the cytopathic impacts on colon carcinoma cell line (Caco-2 cells) initiated by these toxins in human **(Redondo *et al.*, 2017)**. Recently, particular-IgY discovery has been identified with utilization of new adjuvants. For example, article demonstrated a strategy to the use of the IgY-application towards *C.difficile* toxins and/or spores after being utilized in the adjuvant Montanide ISA-70™. In this trial, IgY achieved a better alternative for keeping the animal welfare issue and obtaining the utilization of classical Freund immunomodulators **(Maiti, 2015)**. The clinical signs of patients of resistance to antibiotics for infection caused by *C. difficile*, and then patients are improved after 10 days of oral administration of IgY-powder. Altogether, it would promise positive outcomes of the study which is planned to be finalized in mid-2022.

**IgY can treat nutritional-and/or metabolic diseases:**

**E**ffective utilization of IgY-molecule towards numerous nutritional-and/or metabolic diseases. For example, hypercholesterolemia, hyperphosphatemia, obesity, and celiac disease, had likewise been explored. Hypercholesterolemia positively elevated in circulatory-cholesterol caused atherosclerosis, and consequently leaded to the cardiovascular infections **(Ajufo and Rader, 2016)**. Food-and life-styles are rich in lipids, and therefore have hazard factors revealed a reference to the genetic background. Moreover, the current medications decreasing the cholesterol levels created based on the healthy nutrition and daily activities, besides a preventive therapy provided to diminish the cholesterol levels **(Klein-Szanto and Bassi, 2019)**. New study show that IgY can combat towards sequences of a cholesterol transport protein (Niemann-Pick C1-Like 1) **(Jang *et al.*, 2011)**. It has the immuno-reactivity of particular-IgY and detected the impact of IgY on cholesterol uptake. This uptake of cholesterol is identical to the one of *Ezetimibe*, a traditional administration utilized as a cholesterol uptake inhibitor. In mice, it is announced that IgY used to combat Niemann-Pick C1-Like 1 is fit for improvement on clinical signs of fatty liver syndrome **(Bae *et al.*, 2017)**.

Similarly, it is stated the role of IgY was to combat transport protein (sodium-subordinate phosphate co-transporter-2b) and to prompt an abundant decrease in the phosphate transport Caco-2 cells of human. Considerably, it revealed the effective therapeutic use for the hyperpho-sphatemia **(Hellestad *et al.*, 2015)**. Other study confirmed the role of particular-IgY towards gastrokine-1 **(Boone, 2014)**; a gastric protein secures the gastrointestinal tract **(Paski *et al.*, 2015)**, for the leanliness cases in the mammals. Likewise, it is assessed that the inactivation of the *lipase enzyme* via a particular-IgY in an obese-murine model resulted from fat-induced diet regimens **(Hirose *et al.*, 2013)**. *Lipase* decreased the total hepatic lipids, triglycerides and cholesterol besides a less adiposity in all the body after thirty days of treatment with administration of 2 g IgY/kg per day. As referenced, IgY-structures had likewise been investigated to prevent celiac infection; chronic and/or autoimmune disorders resulted from the intolerance to dietary gluten and consequently associated with *prolamins* **(Skriner, 2013)**. Nowadays, avoiding the clinical signs of this nutritional/metabolic disease is keeping essential for health and long life. Consequently, in this regard, diverse treatment protocols, including inactivation of toxin peptides with inhibiting the effects of antibodies, had been assessed.

Moreover, a chemical structure having entire IgY like IgY Fab-fragments used for the close attachments to celiac disease-related molecules, as evidenced in tissue *transglutaminase type 2* and gliadin in human. The latest discovery likewise covered neither the utilization of various polypeptide sequences of these molecules to inoculate chickens nor the binding-affinity of IgY ($\leq$ 500 nM) **(Skriner, 2013)**. Then, it is reported that capsules containing IgY-molecule giving to ten patients can combat *gliadin* and achieved the biosafety benefits. The same author also confirmed that

one g of oral-IgY given to individuals during eating meals achieved the biosafety levels **(Sample *et al.*, 2017)**. Biosafety pathways are unknown, a randomized, double-blind, crossover experiments is performed, with a supposed involvement of one hundred and fifty patients, which was planned to be finalized by the end of 2022. Moreover, it had been exhibited that IgY-fragments, for example; scFv may likewise had the opportunity to perceive peptic-tryptic digestion gliadin in celiac disease **(Stadlmann *et al.*, 2015)**. However, further experiments are demanded to evaluate the therapeutic influence on IgY towards gliadin and/or tissue *transglutaminase-2* compound.

**IgY can treat chronic immunological manifestations:**

**C**hronic diseases are multifactorial causes that affect the performance of the immune system **(Bagatini *et al.*, 2017)**. Chronic pain syndromes caused by the unknown activation mechanism of inflammatory monocytes, cluster of differentiation $14^{+}$, ($CD14^{+}$) in circulatory-IgY **(Ritz *et al.*, 2011)**. For instance, the patent number: WO2016166246 described a novel therapy depending on IgY-molecule towards the gram-negative bacteria and/or their LPSs ended by discovery of fifteen specific-biomarkers **(Sprotte and Waaga-Gasser, 2016)**. It revealed a therapy for thirty-eight patients with an estimated duration and frequency of pain history for twelve years. On the initial fourteen days, those patients given 2.5 g of IgY/day regularly accompanied with doubling of the dose during the next fourteen days. This particular-IgY succeeded in relief half number of the participants in a regard for dose-dependent. Meanwhile, a study examined the efficiency of IgY towards *E.coli* F18ab and *Salmonella typhimurium* in participants experiencing chronic pains whether associated with fibromyalgia. Likewise, the IgY had additionally been involved for the patent number: WO2012136534, combating LPS, consequently treating periarthritis **(Wesjohann *et al.*, 2012)**, and portrayed an effective relief from symptoms linked to idiopathic periarthritis after administration of 5 g of IgY daily for one week.

Decreasing the side effects is identified after the IgY-administrations with a gradual diminishing of inflammatory cells and proinflammatory cytokines **(Ma and Zhang, 2011; Zhou and Ma, 2018; Zhou *et al.*, 2018)**. Moreover, IgY can reduce the severity of psoriasis symptoms which activates the squamous lesions **(Chapman and El Miedany, 2017)**. Furthermore, the researcher found a new strategy for the therapeutics of psoriasis using specific-IgYs **(Constantin *et al.*, 2017)**. A technique to include the specific-IgY inactivated with cultured cells from psoriatic sores as well as Quillaja saponaria-21 saponin as an adjuvant. It is related to various dosages of specific-IgY such as 20 $\mu$g/mL, 50 $\mu$g/mL and 30 $\mu$g/mL, for liquid, spray, and gel form, respectively. Furthermore, a forty-five, 12 months old male patients, who dealt with an administration of uncooked-egg extracts from immunized hens after topical applications of a gel form containing purified-IgY. After three weeks of the treatment, skin allergic conditions and inflammatory signs stopped, and therefore the pores in the skin tissues are relieved hence the silver-white scales began to fall. In addition, atopic dermatitis causes a severed pruritic-inflammation of the skin in its chronic stage **(Weidinger and Novak, 2016)**. Healing steps consisted of a long course of epidermal barrier repaired to emollients, blocking of individual trigger factors, and anti-inflammatory drugs. The studies found that the IgY-content collected from ostrich's eggs combated lysed *S.aureus* cells **(Tsukamoto, 2013)**.

Moreover, it revealed the bactericidal effects of IgY and/or the findings of a petrolatum-containing particular-IgY (50 $\mu$g/mL). After topically applied to IgY-gel onto *S. aureus*-inflamed regions (twice/day for two weeks), regeneration of skin tissue and relieving of dermatitis signs were evidenced in 49 out of 67 patients (73% recovery in total). Similarly, different researches referred to the influence of specific-IgY on the inactivation of *S. aureus* replications *in vitro* as well as *in vivo* **(Guimarães *et al.*, 2009; Zhen *et al.*, 2009; Hussein *et al.*, 2020; Rehan *et al.*,2020)**. Numerous researches had also investigated the usage of IgY for the remedy of contamination with *Pseudomonas aeruginosa* infection resulted in high mortality in patients affected with cystic fibrosis (CF) **(Davies, 2002)** while the antibiotics had proved to be useless **(Breidenstein, 2011)**. This intervention approach is especially depended on the administrated-IgY to combat *P.aeruginosa* via gargling method, and consequently to permit their neutralization in the respiratory tract.

Moreover, the European patent using IgY against *P.aeruginosa*; European legislation offered several years of marketplace after the approval of this novel IgY-product **(Kollberg, 2003; European Medicines Agency, 2009)**. Researchers finished first and second stages of the study using mouthwash mixed with particular-IgY daily for the therapeutic agents of *P.aeruginosa* infections, with no complications **(Kollberg, 2003; Nilsson, 2007)**. As well, it is revealed that the activity of IgY towards *P.aeruginosa* within the saliva and/or oropharynx after gargling remedy. They recommended that particular-IgY can treat *P.aeruginosa* infection and consequently to prevented pneumonia **(Carlander, 2002)**. Nowadays, it is showed that paticular-IgY can treat *P.aeruginosa* infection and subsequent can be internalized by polymorphonuclear neutrophils **(Thomsen *et al.*, 2015)**. In murine model, specific-IgY had a positive influence on the inhibition of *P.aeruginosa* in

concerning pneumonia **(Vega *et al.*, 2012)**. Moreover, a decrease of bacterial burden of the airways is detected in pigs **(Otterbeck *et al.*, 2019)**. A study investigated the infection of *P.aeruginosa* is recurrent in the sputum samples of those individuals requested to gargle with specific-IgY. As a result, it is confirmed an amazing tolerance profile for the Igs; however, there is no active healing benefit of the anti-*P.aeruginosa* IgY-therapy **(Schuster and Bend, 2018)**. It is evidenced that administration of a non-specific IgY can treat the *P.aeruginosa* infection. Also, it became to be a true that the non-particular IgY had some unspecific inhibitory impacts on the cyctic fibrosia patients complicated with *P.aeruginosa*.

## IgY can overcome neoplasms:

Barrett's esophagus disorder includes the metaplastic alteration in the mucosal cells. It can be changed from the stratified squamous-to the simple columnar-epithelium form, giving the initial step of adenocarcinoma **(Mansour *et al.*, 2016)**. Also, a study claimed a therapy for Barrett's esophageal disease through the IgY-technology towards the sequences of peptide of the extracellular area of such receptors (e.g., ephrin and B4 *tyrosine kinase*) **(Scamell, 2013)**. This patent described that daily oral-IgY administration ended by healing, which may calm down the signs of inflammation but with no significant difference. However, it is reported that rabbit and/or avian polyclonal antibodies targeted extracellular signaling can be initiated *in vitro* anti-cancer defenses/ responses **(Stephenson *et al.*, 2015; Amirijavid *et al.*, 2016)**.

Moreover, ligand-aimed a remedy of cancer had a possibility to induce the multiplication of cells and therefore increased the danger of normal cells. It included the coupling of therapeutics to antibodies and the other ligands identified cancer-related antigens **(Allen, 2002)**. Now, we know that an immunotoxin complicated with IgY towards $CD133^+$ coupled for a type-2-ribosome-inactivating protein originated from the plant *Abrus precatorius*. It is controlled by the multiplication growth of cancer cells in brain tissues **(Pineda Olvera *et al.*, 2015)**. As well, the application stated that IgY-immunotoxin initiated $CD133^+$ cell degeneration in glioma samples of no influences on the other normal related-$CD133^+$ cells. It is hypothesized that this IgY-composition entered to the cancer cells with the aid of delivery-mechanism **(Chavez-Cortez *et al.*, 2019)**. Likewise, other researchers mentioned that the development of immunotoxins sing unique antibodies, both genetically- or chemically-methods, is related to a selected ligand **(Derocq *et al.*, 1988; Liu *et al.*, 2012; Kollmorgen *et al.*, 2017)**. For instance, it is reported that the efficiency of carbon nanotubes conjugated to IgY towards human epidermal growth factor receptor 2 (HER2), is defined in sever breast cancers **(Xiao *et al.*, 2009)**. The latest authors effectively used the *in vitro* results to detect the degeneration of the expression of $HER_2$- in breast-tumored cells.

**Multieffects of specific-IgY toward infection:**

Immunization of chickens with specific bacterial-antigens like *Salmonella spp*. can ultimately target a specific-IgY towards the induced-antigen. However, IgY-molecule had been involved commonly in prognosis of several immunological diseases due to its high stability and functional characteristics **(Amro *et al.*, 2018)**. The efficiency of specific-IgY on killing *Salmonellosis* in chickens had been reported **(Hussein *et al.*, 2020; Rehan *et al.*, 2020)**. Also, the anti-*Salmonellosis* IgYs exhibited a high identity to their matching immunogenicity in quail, suggesting its potentiality to eradicate enterobacterial microbes. As well, the oral-IgYs represented a potential natural alternative to an inhalation of gastrointestinal microbes as *S.typhimurium* and *Salmonella enteritidis* for keeping the human's/animal's health as well as improving food industry **(Esmailnejad *et al.*, 2019)**. IgY-technology gave an evidence for combating *P.aeruginosa* infections that causes a major nosocomial pathogen in acute form along with antibiotic resistance. Moreover, *P. aeruginosa* V-antigen (PcrV) is a vital protein, blocks the pathway to pathogens shapes the type-III secretion system of *P.aeruginosa*, and thus it can be used as a therapeutic target for neutralizing. Nowadays, a recombinant PcrV was used for increasing the titer of particular-IgY antibodies. These IgY-antibodies represented a preventive therapy in acute respiratory manifestations as well as skin burns. Furthermore, IgY anti-PcrV has augmented opsonization capacity and anti-bacterial activity of the host cells **(Ranjbar *et al.*, 2019)**.

The augmentation of phagocytic activity through the IgY-molecule is emphasized *in vitro* causing *P.aeruginosa* in those individuals of CF-disease. By which, IgYs toward *P. aeruginosa* fulfilled their functions by opsonizing the microbe. Therefore, initiating the neutrophils respiratory burst besides the bacterial killing was noticed. Anti-*P.aeruginosa* IgY gave the prophylaxis effects to shape the innate immunity of those CF's individuals in order to help the host neutrophils, and consequently to diminish the bacterial loads **(Thomsen *et al.*, 2016)**. In CF, the pulmonary failure was commonly caused by the *P.aeruginosa* biofilm in its chronic form, which closely activated neutrophils in order to sustain such inflammation. It is indicated that IgY enhanced the beneficial bacteria to do their hydrophobic-action and therefore, neutrophils can engulf the bacteria through an immunological process named "phagocytosis"

**(Amirijavid *et al.,* 2016)**. Passive immunization using IgY finished the first step of airway settlement of *P.aeruginosa* in those patients suffered from CF. Therefore, oral-IgY diminished the bacterial burden of one day after *P. aeruginosa* infection along with improving the clinical symptoms of BALB/c murine model. As well, inflammatory cytokines are decreased, suggesting that anti-*P.aeruginosa* IgY as an antibiotics-adjuvant for diminishing the primary colonization of lungs **(Thomsen *et al.,* 2016)**. Meanwhile, IgY can be used in prognosis of parasitic diseases through obtaining polyclonal-IgY as anti-parasitic therapy. Although other biological researches are indispensable and giving a mAb, anti-parasitic IgY-antigens, is investigated, and it is evidenced that IgY could help for passive immunizations and parasitic-therapy **(Thirumalai, 2019)**.

**IgY is a potential alternative to antibiotics:**

Due to the fact that IgY-technology produces high quantity of IgY-antibodies along with its cost-effective extraction from egg-yolk. It is considered an effective opportunity to produce antibiotics to combat disease/infection, and therefore IgY-technology, as antiviral drug, makes sense of researchers, physicians and pharmacists for using in the applied field of virology **(Brelje *et al.,* 2002; Schade *et al.,* 2005; Hussein *et al.,* 2020; Rehan *et al.,* 2020)**.

***"IgY-related influenza-and Corona-viruses":***

IgY is promising a potential biomarker of respiratory viral infection. IgY could be associated with viral N-protein of *influenza virus* in pigs, and identified as exerting a master in viral multiplication. Thus, the efficiency of IgY-molecule is confirmed for diagnosis and therapeutics of *influenza virus* **(Eisfeld *et al.,* 2015; da Silva *et al.,* 2018)**. Currently, the Iceni Diagnostics products can determine a single-virus; however, the next generation of diagnostics can detect a series with differential diagnosis to similar symptoms of pathogens. Therefore, it would be a good possibility to differentiate between *flu* and COVID-19 in a single sample. Furthermore the interaction of the SARS-2 CoVs its glycoproteins-receptor makes it seasonally consistent, so, even if the virus genetic code mutates, it will be identified effectively **(Baraniuk, 2020)**. However, CoVs were recognized previously as the main causative agent of viral respiratory diseases, exclusively in vertebrates. Further, the severe acute respiratory syndrome (SARS)-CoV originated from China causes a global challenge of unexpected recovery **(Perlman and Netland, 2009)**.

The CoV is quickly spreading worldwide, causing around 10,000 infection records giving 10% mortality **(Drosten *et al.,* 2003; Rota *et al.,* 2003)**. Hence, the SARS started from China, passive immunization using plasma taken from those recovered Chinese patients is the best choice for therapy **(Fu *et al.,* 2006)**. Meanwhile, the latest researchers reported that IgY-preparations are ultimately targeted with anti-SARS-CoVs influences after being isolated from egg-yolk of immunized chickens on antigens of SARS-CoVs. Also, they stated that the targeted-IgY product had high purity as well as high biological activities. The purified-IgY neutralized the SARS-CoVs. Moreover, the prepared-IgY is constant when being lyophilized, giving the product good manufacturing qualities **(Fu *et al.,* 2006**). Therefore, anti-SARS IgY is promising a targeted therapy for SARS-CoVs. In the outbreaks of CoVs, a prompt intervention can be activated to produce IgY-antibodies. This IgY-production may take over forty-five days from hens' vaccination to the completion.

Thus, till the vaccination for the outbreak of CoVs is put in use, IgY-applications can fill the gap of patient's immunological needs. Even a proper vaccination was established, passive immunizations can duplicate the possibility for the prevention of the COVID-19 pandemic **(Fu *et al.,* 2006)**. In 2007, a post-SARS outbreak has been identified as a biological weapon waiting to explode **(Cheng *et al.,* 2007)**. On 2020, we are threatened by the biggest COVID-pandemic since the *Spanish-Flu* pandemic by 1918. Hence, the biomedical sciences are in progress worldwide, scientific information and findings change almost in each hour. Now, there are 44 products announced/ discovered in clinical trials for preventing COVID **(Gavi, 2020)**. However, the potential discovery might be generated on the mid-2020. Nevertheless, until vaccination is being applied and generated, there are several considerations to be thought. However, alternative protocols to prevent CoVs infection and to treat the infected individuals-related, are essentially demanded. Passive immunization, given by plasma from recovered patients, was already approved of USA **(Mail Online, 2020)**. Furthermore, SARS-CoV-2 (shown in **Figure 3**) entered the host cells through the attachment to ACE2, accompanied with the functional role of primers, TMPRSS2.

The expression level of ACE2 and TMPRSS2 across the cells in lung (12 donors, 39,778 cells) besides, in subsegmental-bronchial branches (4 donors, 17,521 cells) using single-nuclei and single-cell RNA-sequencing, respectively. While TMPRSS2 was mainly raised in tissues and in the subsegmental-bronchial branches, ACE2 was abundantly raised in a transient-secretory cell type. These transiently of cells revealed the effective mechanism of *Ras homologous guanosine triphosphate enzyme* (RHO GTPase) and viral access to the host cells indicating the higher possibility for SARS-CoV-2 infections, which predicting the occurrence of COVID-19 infection and its pathogenesis

**(Lukassen *et al.*, 2020)**. Then, the S-proteins bound to cells, particularly SARS-CoV-2 of human cells to start the viral infection. Focusing of the mode of action of S-proteins is demanded to find the weakness points and help us to find the way for combating COVID-19 **(Baraniuk, 2020)**. Also, it might enable us to block the action of *Furin*-protein **(Baraniuk, 2020)**, because *Furin* helped the two subunits of the S-proteins to make a separation, and consequently permitted the virus to penetrate and access to the host cells. This pathway to *Furin* was commonly occurred in the human body which revealed the high possibility of SARS-CoV-2 infections. Interestingly, the molecule separated *Furin* from the CoV made an inhibition to its pathway.

Thus, the researchers nowadays are evaluating whether the *Furin* blockers can make such action. Peptide-blockers toward the SARS-CoV-2 caused a global pandemic infection. Also, they were formed into the two sequential self-supporting α-helices derived from the *protease* domain of ACE2, which attached to the SARS-CoV-2 RBD. Molecular dynamical approaches confirmed that the α-helical peptides can keep their secondary structure and give an inhibition to SARS-CoV-2 infection. Additionally, several peptides attached to the outer surfaces of nanoparticle carriers to indicate a multivalent binding to the SARS-CoV-2 receptors. The predicted peptide blockers can offer easy and potential therapeutic towards the COVID-19 pandemic **(Han and Král, 2020)**. Hence fifty percent of all viral proteins are glycosylated; CoV has some glycosylated binding sites **(Watanab *et al.*, 2019)**. Meanwhile, glycosylation site of S.protein is essential for promoting COVID-19 infection. It is noticeable that glycosylation profile on SARS-CoVs is characterized by high-resolution LC-MS⁄MS about *N*-glycosylation sites were found occupied out of 22 potential sites along with two *O*-glycosylation sites bearing core-1 type *O*-glycan. Some *N*-glycosylation sites were partially glycosylated. Therefore, glycosylation of S.protein of CoV has important role in COVID infection progress. It means inhibition of the glycosylation pathway of CoV S.protein would be helpful to block the pathway of ACE2-receptors and prevent SARS-CoV-2 infection.

## Future Perspectives

Egyptian team revealed that IgY-based composition mixed during feed formulation **(Rehan *et al.*, 2020)** can be used as antibiotic alternatives to gram-positive/negative bacteria **(Hussein *et al.*, 2020)**. For the egg-yolk pharmaceutical purposes, the extracted-IgY is considered an essential step. Bioactivity and quality control of IgY are demanded for the heavily production of antibodies. The oral administration of IgY seem to be the most cost-effective protocol. However, the parenteral administration of IgY is more restricted due to dose-, age-, case-dependent history and therefore, oral administration is preferable to use **(Hussein *et al.*, 2020; Rehan *et al.*, 2020)**. In reality, the researches focused on parenteral administration of IgY are scarce due to the evidence of side effects during a parenteral administration of mammalian immunoglobulins in humans. Therefore, the researchers concluded that the biosafety is demanded. Hence, viruses causing pandemics such as COVID-19, we have to draw the future perspective of the most potential therapeutics and vaccine development. Large‑scale and research collaborations are demanded to protect the health of human population **(Docea *et al.*, 2020; Tsitoura *et al.*, 2020)**. Therefore, it would be great to join these efforts, and opening new gates to use passive immunized-IgY intensively as adjuvant viral-therapy in RTI. To proceed further, we have to clearly understand the way to increase the IgY-production and purity yields to improving the targeted therapies. It is well-known that the S-protein is the co-factor of the CoV to enter to the host **(Li, 2016)**, followed by the initiation of the host's immune responses through the ACE2-receptor **(Watanabe *et al.*, 2019)**.

Interestingly, we have to notice here that chicken-IgY has a specific-monoclonality, and thus it has better binding-affinity compared to the ones obtained from immunizing mammals **(Lee *et al.*, 2017)**. Unlike to IgGs, having IgY‑scFv that is easier as these fragments involved in disease prognosis, and block the attachment of CoVs to the ACE2-receptors **(Spillner *et al.*, 2012; Zhang *et al.*, 2017)**. Consequently, IgY‑scFv would give the opportunity of CoVs to adapt the ACE2-receptors and combating for SARS-CoV-2 infection. Using IgY-molecule in viruses of respiratory manifestations is promising a potential therapy to infectious diseases. It is not a surprise that the IgY‑technology is a new frontier for medical researches because its effective role in both human as well as animal health. In particular, the functional properties of IgY structure give them a recommended target for the biotechnological researches, diagnostics, and therapeutics. The main advantage of IgYs are being a natural extracts from egg-yolk through a non-invasive protocol, and therefore used as alternative for obtaining mammalian antibodies. Therefore, the researches focused on IgY on bacteria, virus and cancer were numerous. Hence, specific-IgY has already been tested as anti-viral therapy in several studies, the actual COVID-19 pandemic context, and SARS need extensive researches on therapeutic approaches to be exploited in detail.

## Limitations

Although, this review provided a detailed overview of chicken egg-yolk IgY-product for

combating pathogenic infections, we have to apply to confirm the effects of IgY-product on CoVs infection *in vivo*.

## CONCLUSION

This review provided a detailed overview of chicken egg-yolk IgY-product for combating pathogenic infections; particularly the novel complicated CoVs infection. Interestingly, the egg-yolk antibody has the novelty, biosafety, and efficiency to be used as a targeted therapeutic and prophylaxis of multidisciplinary lines of pathogens. The CoVs S-protein along with ACE2 can regulate the viral access to host and assist the CoVs-gene mutations. However, the anti-SARS CoV-2 scFv IgY-antibodies blocked the tagging of CoVs to the host cell ACE2-receptors through the passive immunization process. Therefore, we could announce here that IgY is promising by 2021 to be on the top medications list of COVID-19.

## Abbreviations

*ACE2: angiotensin-converting enzyme 2; C.albicans: Candida albicans; C.difficile: Clostridium difficile*; Caco-2: human colon carcinoma cell line; CD: cluster of differentiation; CF: cystic fibrosis; COVID-19: *Coronaviruse* disease-2019; CoVs: *Coronaviruss*; *E.coli: Escherichia coli; E.coli*: *Escherichia coli*; ELISA: *enzyme-linked immunosorbent assay*; E-protein: envelope protein; *H.Pylori: Helicobacter pylori; HE-protein: hemagglutinin-esterase protein;* HER2: human epidermal growth factor receptor 2; IFN: interferon; IgY: immunoglobulin Y; *LPS: lipopolysaccharide*; M-protein: membrane protein; mAbs: monoclonal antibodies; MERS-CoV: middle east respiratory syndrome *Coronaviruses*; N-proteins: nucleocapsid protein; NSP: non-structural protein; NSP12: nonstructural protein; ORF: open-reading frame; *P.aeruginosa: Pseudomonas aeruginosa; P.Gingivalis: Porphyromonas gingivalis;* PcrV: *P.aeruginosa* V-antigen; PEG: polyethylene glycol; pp1: polyproteins; RBD: receptor binding domain; *RHO GTPase: Ras homologous guanosine triphosphate enzyme;* RIT: respiratory tract infection; RNA-dependent RNA polymerase: RdRp; RTC: replication-transcription complexes; *S.aureus: Staphylococcus aureus; S.mutans: Streptococcus mutans; S.typhimurium: Salmonella typhimurium;* S1: subunit1; S2: subunit2; SARS:sever acute respiratory syndrome; ScFv: single-chain variable fragment; S-glycoprotein: Spike (S)-glycoprotein; S-protein: Spike (S); TMPRSS2: transmembrane protease serine 2; VH: variable domains of heavy chain; VL: variable domains of light chain; and N-protein: nucleocapsid protein.

## ACKNOWLEDGEMENTS

We thank the Menofia University for giving us the assistance to finish the work. Moreover, we appreciate Dr. Amr Elkelish's help for editing the review, at the Faculty of Science, Suez Canal University. Furthmore, all figures were made using biorender.com. Also, we deeply appreciate referees' efforts for the handling of our review.

## Declaration of Conflicting Interests

The authors revealed that there was no potential conflicts of interest.

## Authors' Contributions

Ibrahim F. Rehan and Asmaa Elnagar were jointly developed the hypothesis and concept of the study. Ibrahim F. Rehan, and Asmaa Elnagar shared in editing process. They were also involved in drafting and revising the review.

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**How to cite this article:**
**Ibrahim F. Rehan and Asmaa Elnagar, 2021.** Chicken Egg Yolk-IgY: Passive Immunization Promising Targeted Therapy of COVID-19 Pandemic. Journal of Applied Veterinary Sciences, 6 (2): 67 – 91.
DOI: https://dx.doi.org/10.21608/javs.2021.164324