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Review Article
Avian Egg Yolk Antibodies (IgY) and their Potential Therapeutic Applications for Countering Infectious Diseases of Fish and Aquatic Animals

[1]Mohammad Jalil Zorriehzahra, [2]Ruchi Tiwari, [3]Swati Sachan, [4]Kumaragurubaran Karthik, [5]Yashpal Singh Malik, [6]Maryam Dadar, [7]Muhammad Sarwar, [8]Maryam Sayab and [9]Kuldeep Dhama

[1]Department of Aquatic Animal Health and Diseases, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Tehran, Islamic Republic of Iran
[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwa Vidyalaya Evam Go-Anusandhan Sansthan (DUVASU), 281001 Mathura, Uttar Pradesh, India
[3]Immunology Section, ICAR-Indian Veterinary Research Institute (IVRI), 243122 Izatnagar, Uttar Pradesh, India
[4]Division of Bacteriology and Mycology, ICAR-Indian Veterinary Research Institute (IVRI), 243122 Izatnagar, Uttar Pradesh, India
[5]Division of Biological Standardization, ICAR-Indian Veterinary Research Institute (IVRI), 243122 Izatnagar, Uttar Pradesh, India
[6]Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
[7]G.M. College for Science and Technology, Faisalabad, Pakistan
[8]ACE College for Women, Sargodha Road, Faisalabad, Pakistan
[9]Division of Pathology, ICAR-Indian Veterinary Research Institute (IVRI), 243122 Izatnagar, Uttar Pradesh, India

Abstract
In the current scenario of increasing and emerging drug resistance in various microbial pathogens, traditional antibiotics are becoming less effective and thus globally research has focused on developing alternative therapeutic regimens having efficient germ killing abilities. The leading alternatives include use of phages, prebiotics, probiotics, cytokines, avian egg yolk antibodies, toll like receptors, medical herbs and various other immunomodulatory/immunotherapeutic approaches. Out of these valuable therapies, the application of oral passive immunization using avian egg yolk antibodies (Immunoglobulin Y, IgY) offers promising future avenues for designing and developing novel prophylactic and treatment strategies against infectious diseases in both humans and animals, particularly countering the enteric pathogens. Hitherto studies confirm beneficial applications of IgY antibodies in animals (Calves, lamb and goat, cats and dogs), poultry and humans, however such studies in fish and aquatic animals are comparatively less. The present study presents as overview on avian egg antibodies, their salient features, advantages and limitations and then describes the potential therapeutic applications of IgY for the prevention and treatment of infectious diseases of fish and aquatic animal species, as well as speculating the future prospects of upcoming IgY technology. Taking into account the valuable prophylactic and therapeutic applications of IgY antibodies, further explorative research in this area could pave way for designing and developing effective pharmaceuticals and treatment options for various infectious diseases of fish/aquaculture animals as well as for safeguarding health of humans and their companion animals.

Key words: Avian egg antibodies, IgY, egg yolk, fish, aquatic animals, infectious diseases, prophylaxis, therapy

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Corresponding Author: Kuldeep Dhama, Division of Pathology, ICAR-Indian Veterinary Research Institute (IVRI), 243122 Izatnagar, Uttar Pradesh, India

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INTRODUCTION

The emergence of drug resistant microbial pathogens is prompting scientists to search for other germ-killing option like the use of alternative and complementary therapeutic regimens like phages, prebiotics, probiotics, cytokines, avian egg yolk antibodies, toll like receptors, medical herbs and various immunomodulatory/immunotherapeutic approaches to counter infectious diseases of aquatic animals[1-7]. Out of these, avian egg yolk antibodies (EYA) are gaining interest and considerable attention of worldwide researchers of life sciences. Avian egg antibodies constitute a good source of beneficial antibodies as immunized hens could lay large number of eggs in a period of time containing a high concentration of antibodies in their yolk contents[8-11]. The immunologically active proteins and peptides of IgY and its immunomodulatory mechanisms have been recognized[12-14]. The use of transgenic chickens producing antibodies in the egg ovalbumin has provided bioreactor hens as a good source of avian egg antibodies[15,16].

The avian eggs act as efficient repositories for promising antigen-specific antibodies having numerous biomedical applications to be used in immunodiagnostics, proteomics and in immunotherapy for combating infectious diseases[17-20].

Apart from useful treatment options in humans the EYA have proved their potent prophylactic and therapeutic applications in combating several infectious diseases including of bacterial and viral origin in animals and fish/aquatic animals[7,21-28]. Passive immunization using avian egg antibodies (IgY) has been found to be effective in providing protection against several pathogens affecting animals and humans viz., *Helicobacter pylori* in humans, bovine and human rotaviruses, bovine coronavirus, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*, *Staphylococcus* and *Pseudomonas* spp., poultry pathogens such as *Salmonella*, *Campylobacter*, infectious bursal disease (Gumboro) virus and newcastle disease virus; fish and aquatic pathogens like shrimp white spot syndrome virus (WSSV), *Yersinia ruckeri*, *Edwardsiella tarda*, *Aeromonas salmonicida, Vibrio alginolyticus, Vibrio anguillarum, Shewanella putrefaciens* and *Pseudomonas fluorescens*[17,27,29-33]. The IgY acts by enhancing the phagocytic ability macrophages and inhibition of growth and colonization of bacteria[19,31]. Agglutination, adherence-blockade, opsonization, inhibition of enzymatic activity and toxin neutralization are other mechanism of action of IgY[27]. The IgY could also serve as a valuable alternative to antibiotics for treating antibiotic-resistant microbial pathogens. Avian egg yolk antibodies could also be useful in food preservation as natural antimicrobial agents. Since, IgY is suitably stable in saliva, thus can be used to treat certain localized infections related to oral mucosa[19]. Apart from above utilities, IgY can also be employed as growth promoting agent as observed in fish by using antibodies against several neuropeptides of gut such as cholecystokinin and neuropeptide Y[19].

A recent systematic review and meta-analysis demonstrated that the beneficial effects of IgY for prophylaxis and therapeutic purposes. With regards to this, more explorative research studies are needed to be performed utilizing the gold standard animal experiments with an aim to bring into play IgY alone or in combination options with supplementary alternative measures to be adapted[32].

Reports regarding beneficial applications of IgY in fish are very limited as compared to animals (Calves, lamb and goat, cats and dogs), poultry and humans. This study describes the potential immunotherapeutic applications of avian egg yolk antibodies (IgY) for the prevention and treatment of enteric diseases of fish and aquatic animal species along with key features and advantages as well as speculating the future prospects of IgY technology. The information compiled will be beneficial for fish professionals, researchers and pharmaceutical industry to design and develop novel alternative therapeutic and prophylactic module utilizing the tremendous abilities of IgY antibodies for safeguarding health of fish and aquatic animals as well in promoting the various avenues of this promising technology.

POTENT ADVANTAGES OF AVIAN EGG YOLK ANTIBODIES (IgY)

After immunization, specific IgY antibodies are continuously synthesized, excreted into the blood of the hen and then transferred from blood/serum into the egg yolk. The major antibody produced by chickens is immunoglobulin IgY that provides necessary immunity to the progeny chicks in terms of passive humoral immunity required against various pathogens in early days of chicks until complete maturation of their immune system[18].

Laying chickens are used as immunization host for a specific antigen of interest encoding the targeted protein for the generation of specific and biologically active egg yolk immunoglobulins (IgY) which are vertically transmitted from their serum into the egg yolk from where they can be extracted and purified and used as an alternative source of

antibodies for the prevention and treatment of infectious enteric diseases. Laying hens are very efficient producers of specific antibodies. Nearly 280-300 eggs are being laid by a hen in a year with 100-150 mg IgY per egg yolk which could give 20-40 g IgY per year through eggs of a bird[29,34]. Thus an egg can be considered as a small "Factory" for antibody production. Further, use of chicken eggs as source of antibody, reduces the number of experimental animals such as mice, rabbit for research purpose, which is very much required today for the sake of animal welfare and solving the problem of animal ethics issues[34]. Oral administration of IgY have been useful in controlling infectious diseases of bacterial and viral origin[35,36]. Oral administration of IgY possesses many advantages over the use of mammalian IgG including cost-effectiveness, convenience and higher yield[27]. The IgY is non-toxic, without any residual and negative effect to the environment.

Usage of IgY technology has several advantage viz., non-invasive antibody harvesting procedures avoids concerns regarding the welfare of the immunized animals, as the IgY antibodies can be separated without sacrificing chickens, convenience of simple egg collection, rapid and simple IgY isolation from egg yolks, a good alternative to conventional polyclonal antibody production in mammals[37]. Chickens possess numerous advantages over mammals to be used as hosts for immunization, especially their phylogenetic distance, modes of immune diversification and the means by which IgY immunoglobulin is deposited in the egg yolk and showing remarkable high affinity, avidity and highly neutralizing abilities for an infectious pathogen[38]. Also, chicken requires less quantity of antigen to stimulate and produce specific antibodies (approximately 2-10%) than mammals (only about 5%)[29]. The stability of IgY in the oro-gastrointestinal tract and its safety issues are well proven, while the stability of IgY can further be enhanced by encapsulating with alginate microcapsules which protects it from the action of low pH gastric juices[39]. Mammalian sera contains many classes of antibodies after immune stimulation with single antigen whereas egg yolk consist of only single class of antibody i.e., IgY, thus facilitating ease of purification[29]. One-step purification of IgY antibodies in large amounts from egg yolk provides a practical continuous supply, epitope spectrum of IgY potentially facilitates novel specificities, lack of serological cross-reactivity with mammalian epitopes, Fc receptors and complement steer clear of interference during assays and potentiates the outcome of immunological methods adapted as well avoids mediation of inflammatory responses during their usages[17,20,34]. Thus IgY can be used as long term therapeutic agent without any negative effects on animal health[19]. Because IgY are resistant to the gastric barrier, these are of particular interest for passive immunotherapy of gastrointestinal tract infections. Added to these, eggs being normal dietary element with no religious taboo issues so pose practically no risk of toxic side effects of IgY antibodies. However, it is important to analyze the allergic reactions as observed by consumption of egg protein in some individuals[34]. But it has been suggested that the IgY is not associated with any risk of causing allergic reactions[31].

As chickens are less costly, egg yolk antibodies can be produced in a more cost effective and easy manner compared with production of antibodies from mammals and also have preferred advantages in their various applications including usage in immunodiagnostics and immunotherapeutics[18,29]. Unlike mammalian IgG, IgY antibody does not react with rheumatoid factor hence, no false positive result in rheumatoid immunoassay[37]. The IgY preparations in egg yolk powder, purified powder and liquor may offer a practicable and noteworthy applications of avian egg antibodies. It has also been demonstrated that IgY antibodies can be used to prevent rejection in case of xenotransplantation thus, can be looked upon as potential solution of overcoming transplantation failure[37]. Also, IgY can be used to overcome the problem of zoonotic infections such as cryptosporidium infection and bioterrorism[19,34]. Apart from containing IgY, egg yolk contains other anti-infectious agents which gives additional benefit to the patients[18]. Salient advantages of avian egg yolk antibodies (IgY) over the conventional antibodies are depicted in Fig. 1. Figure 1 depicts various advantages of IgY technology over the conventional antibodies. Advantages include: Non invasive antibody harvesting, egg collection easy, cost effective, IgY is non toxic, non allergic etc.

The "DNA-designed" avian IgY antibodies produced via immunization with a gene vector expressing a corresponding antibody *in situ* avian host cells allows direct generation of IgY antibodies from a plasmid DNA that could avoid the cumbersome procedures for obtaining purified antigens in a conventional antibody production system[40]. Use of various adjuvants such as CpG ODN has been found to enhance antibody production with DNA vaccines[41]. The IgY obtained by DNA immunization has a closely related epitope pattern to that observed in virtual viral infection. A quick and inexpensive production of antibodies will provide pertinent tools to link the fields of genomics and proteomics. However, polyclonal

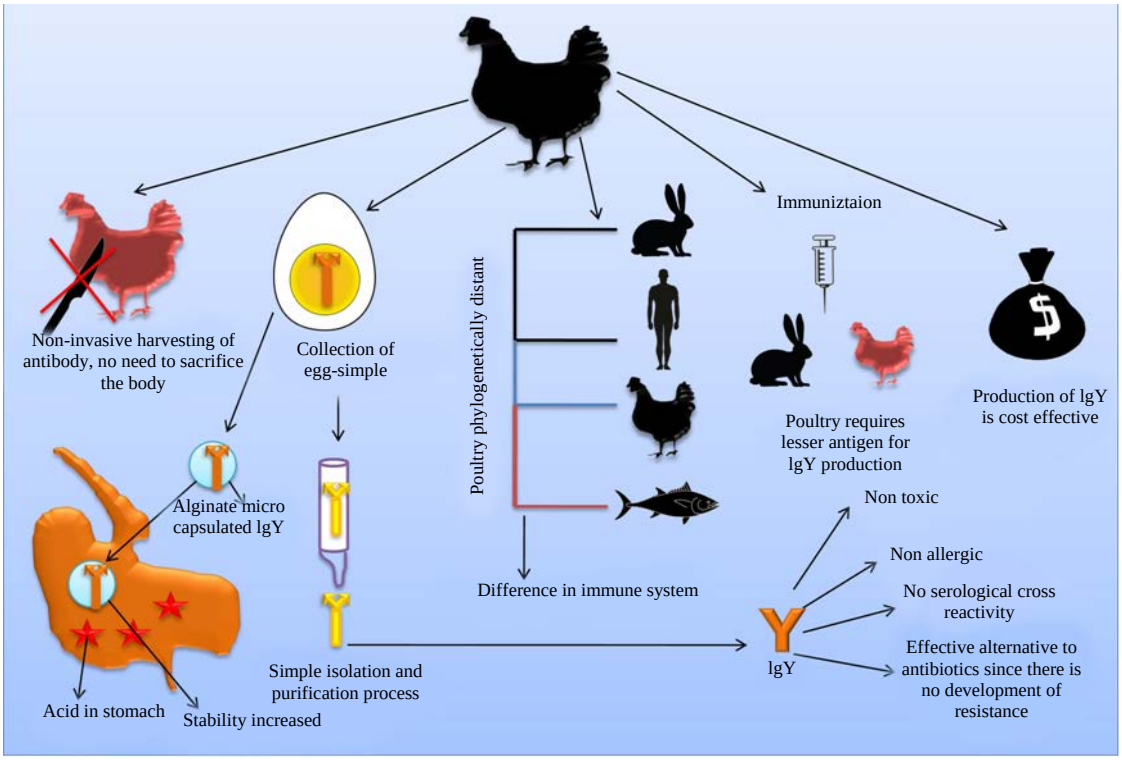

Fig. 1: Advantages of avian egg yolk antibodies (IgY)

nature of IgY antibodies could limit their use. In such circumstances, recombinant IgY can be created from mammalian monoclonal antibodies that would facilitate further utilization of many benefits of the IgY technology[20].

THERAPEUTIC APPLICATIONS AGAINST INFECTIOUS DISEASES OF FISH/AQUATIC ANIMALS

Specific IgY has been found to have effective therapeutic values to control various bacterial and virus pathogens in fish and aquatic animals[21,27]. The EYA has been found effective in diseases like White Spot Disease (WSD) in shrimps and crayfishes, *Vibrio anguillarum* and *Yersinia ruckeri* in rainbow trout, *Aeromonas hydrophila* in polyploid gibel carps (*Carassius auratus gibelio*), Edwardsiellosis in Japanese eel (*Anguilla japonica*)[23,41-47].

Chicken IgY, against a truncated fusion protein of VP28 and VP19, have been found to neutralize WSSV which is an economically important pathogen causing high mortality and losses to cultured shrimp industry worldwide[44]. The IgY antibodies recovered from the egg yolk of chickens immunized with a DNA vaccine constituted with structural proteins genes of WSSV or with inactivated WSSV have been shown to demonstrate utility in passive immunization of shrimp by possessing a high affinity and neutralization activity for WSSV, good survival rate of experimentally challenged shrimps, particularly using the traditional inactivated vaccine[36]. The immunization with specific IgY antibodies was found protective against this viral infection when administered through intramuscular injection, oral and immersion application in crayfish (*Procambius clarkiaii*) challenged with WSSV[36]. Thus prospective immunotherapeutic role of specific IgY in preventing this important infection in shrimp has been suggested.

Passive immunization with IgY has also been reported to be effective against *Yersinia ruckeri,* the causative agent of enteric redmouth disease (systemic bacterial septicaemia) affecting rainbow trout (*Oncorhynchus mykiss*); immunized fishes revealed lower mortality rate and fewer infections[43].

Efficacy of EYA have been studied against *Edwardsiella tarda* infection, an important fish pathogen which spreads through the intestinal mucosa and IgY were found protective in preventing this infectious disease of fishes[21,42]. In a limited trial, specific IgY antibodies when simultaneously administered orally to Japanese eels with the *E. tarda* resulted in a good survival rate and with no disease symptoms

seen in IgY administered eels, which indicates an effective role of anti-*E. tarda* IgY in preventing edwardsiellosis[42].

Li *et al.*[45] showed that specific IgY immunoglobulin obtained from egg yolk of immunized white leghorn hens immunized against formalin inactivated *Aeromonas hydrophila* could provide protection rate of 60-70% in polyploid gibel carps. The ELISA confirmed that hyperimmune serum shows maximum IgY level 56th day post-immunization and peak level was maintained till day 133th. Similarly, in another study outcomes advocated that passive immunization of ayu, *Plecoglossus altivelis* by oral administration with pathogen-specific chicken IgY is a valuable means for controlling *Vibrio anguillarum* infection in ayu. Treatment with specific IgY isolated from egg yolks of hens immunized with formalin-inactivated *V. anguillarum* significantly enhanced the phagocytic activity of macrophages for *V. anguillarum* in the presence of specific IgY and decreased levels of tumor necrosis factor-α (PaTNF-α), interleukin-1β (PaIL-1β), transforming growth factor-β (PaTGF-β) and leukocyte cell-derived chemotaxin-2 (PaLECT2) and proved protective effect of IgY against *V. anguillarum* infection in the ayu[35].

The IgY could be a useful strategy for the treatment of piscirickettsiosis against intracellular bacterial pathogen *Piscirickettsia salmonis* of fish when fishes were treated orally. Specific IgY was produced by immunizing hens with *P. salmonis* proteins and purified from egg yolks by ammonium sulphate precipitation method. Specific IgY effectively inhibited the growth of *P. salmonis* by agglutination where IgY interacted with surface components of the pathogen. *In vitro* and *in vivo* studies showed that anti-*P. salmonis* IgY antibody reacted strongly and particularly against *P. salmonis* proteins with a positive protective effect[48].

For passive immunization of gastric rainbow trout (*Oncorhynchus mykiss*) and a gastric common carp (*Cyprinus carpio*) when IgY antibodies were administered orally their protective potential was found higher as they can cross the gastric barriers to maintain the plasma level in the body[49,50]. Similarly, passive immunization of ornamental koi carp induced by immersing fish into aquarium water containing specific anti-*Aeromonas salmonicida* IgY provided significant protection against *A. salmonicida* infection with reduced clinical signs of skin ulceration and disfiguring ornamental fish[51]. Hence, use of chicken IgY in the ornamental fish industry is a novel means of prospective prophylaxis against pathogens attacking the skin and gills of fishes from cohabitation infection by immersing fishes into specific IgY containing rearing water. The *V. alginolyticus* can cause huge economic loss by causing death of small abalone. The IgY against *V. alginolyticus* was encapsulated with alginate and it was incorporated into basal diet of small abalone at 5, 10% and a control group without IgY treatment was also included in the study. Alginate encapsulation increased the stability of IgY in the gastrointestinal tract of the shellfish. IgY treated group had a survivability ranging from 65-70% post challenge after 14 days with 1×10 CFU[39]. Other researcher through separated studies confirmed that specific immunoglobulins from hen egg yolk are promising novel alternative approach for disease control in fishes caused by variety of microorganism[39,49,51] *Vibrio splendidus* is an importnat pathogen causing skin ulceration syndrome in sea cucumber (*Apostichopus japonicus*). In a recent study, IgY against *V. splendidus* was produced by two step salt precipitation and ultrafiltration in white leghorn chicken. Purity of IgY was 83% and its was injected intraperitoneally in sea cucumber at a concentration of 10 mg mL^{-1} or sea cucumber's were immersed in IgY of 1 mg mL^{-1}. Survival of the tested sea cucumbers was analyzed and it was found that intraperitoneal injection resulted in 80% protection while immersion resulted in 75% protection. Similarly the burden of bacteria in IgY treated sea cucumber was also less in the respiratory tract and intestine when compared with untreated control. Thus this study revealed the potential of IgY against *V. splendidus* infection[52].

Specific IgY displayed significant antimicrobial activity to prolong the shelf life of refrigerated fish and prevent spoilage of fishery food from specific spoilage organisms (SSO) such as *Shewanella putrefaciens* and *Pseudomonas fluorescens* under aerobic chilling storage conditions and now antigen-binding fragment plus the hinge region (IgY-Fab') is also considered a promising tool for improving the efficiency of natural antimicrobial agents[53]. The IgY-Fab' demonstrated higher and more durable antimicrobial potential by effectively inhibiting the bacterial growth and prolonging the shelf life of marine products more efficiently as a novel strategy for fish preservation. Significant broad spectrum antimicrobial activity of the anti-SSO IgY extended the shelf life of refrigerated fish products from 9-15 days in the presence of the specific IgY and demonstrated its bio-preservative potential for seafood and marine products[54]. Potential therapeutic application of IgY in countering infectious diseases of fish and aquaculture animals is presented in Fig. 2. The WSSV recombinant protein or DNA vaccine of WSSV

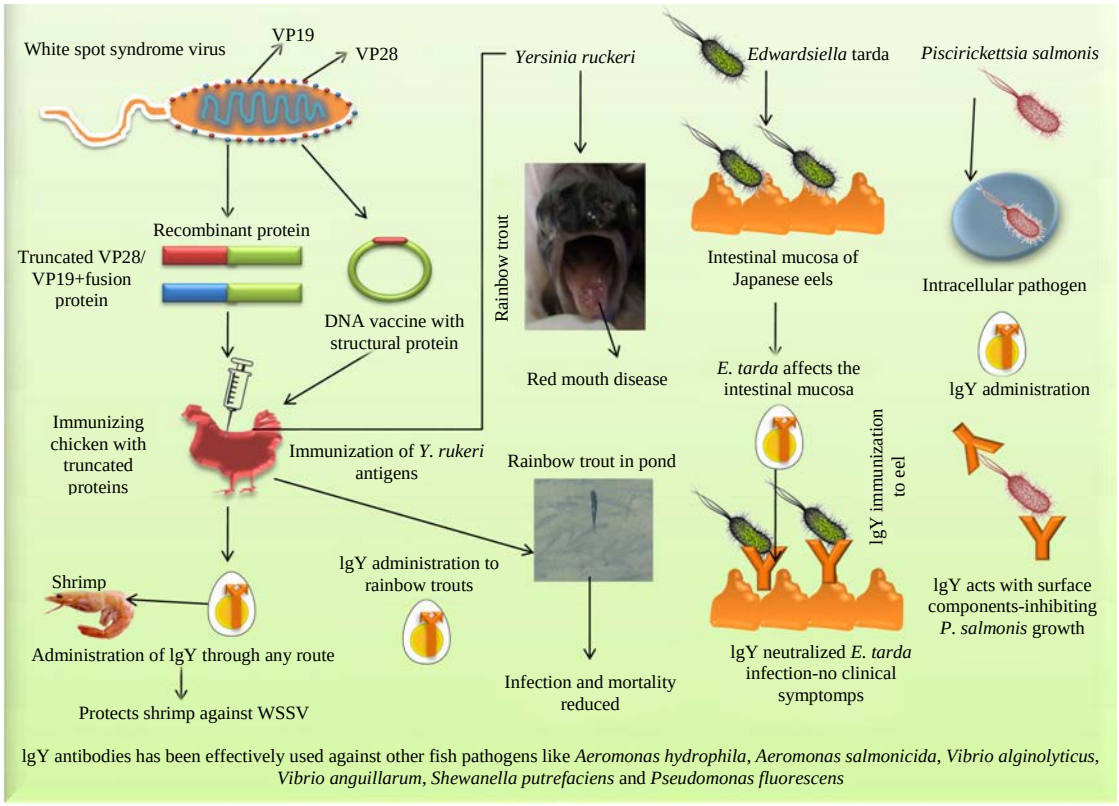

Fig. 2: Use of IgY in aquaculture

is used to raise anti WSSV IgY which when administered to shrimp and crayfish, protects from WSD. *Yersinia ruckeri* causes red mouth disease in rainbow trout. IgY against *Y. rukeri* reduces infection and mortality caused by *Y. rukeri*. *E. tarda* infects intestinal mucosa of Japanese eel. Administration of anti *E. tarda* IgY neutralizes the bacteria and eliminates the symptoms. Similarly, IgY antibodies has been used with good results against various pathogens like *Piscirickettsia salmonis, Aeromonas hydrophila, Aeromonas salmonicida, Vibrio alginolyticus*, *Vibrio anguillarum*, *Shewanella putrefaciens* and *Pseudomonas fluorescens* (Fig. 2).

POTENTIAL LIMITATIONS OF IgY

Numerous advantages support the use of IgY in human and veterinary field, however there are some shortfalls that needs to be strengthened so that this technology can be used to great success. Major drawback is the proteolytic disintegration of IgY in gastric conditions when administrated through oral route. Studies conducted in eels showed that IgY could resist the digestive environment but when the pH is less than 3, activity of IgY is completely lost[21,55,56]. Small intestine is the area of absorption of IgY but they do not resist the acidic environment of the stomach. Hence methods that can protect the IgY from acidic pH and carry towards the site of absorption is essential. One such method is encapsulation using alginate or any other method can prevent the IgY from acidic pH[39]. This method seems to be a better option for preventing the proteolysis but they are cost effective hence it is difficult to employ at commercial level. Studies also report that IgY can be used as prophylactic agent against specific bacterial infection rather than as a therapeutic agent[42].

CONCLUSION AND FUTURE PROSPECTS

The tremendous potential of avian eggs as valuable repository of promising immunotherapeutic antibodies (IgY) needs to be explored to their full potential against harmful effects of various infectious pathogens and safeguard health of viable fish/aquatic animals. Increasing problems of emerging bacterial resistance issue and the presence of drug residues in fish products poses food safety and public health concerns. In this scenario, EYA could provide a viable option due its potent therapeutic avenues. Application of EYA

immunization is a labor intensive procedure and might have limited applications in commercial aquaculture conditions and fishes/aquatic animal species raised in the ocean. The oral administration of specific IgY to counter fish pathogens could provide a valuable alternative method to antibiotics/chemotherapy for prevention of diseases in fish farms. Thus, oral IgY antibodies could offer very promising possibilities for useful passive immunization strategies in the years to come and added to this the usage of IgY as feed additive and immersion solutions may give rise to useful practicable methods for its potent applications to be implemented effectively in prevention and control of infectious diseases of fish. Various laboratory studies and clinical trials of IgY supplemented foods for disease prevention and control purposes have been reported. It is a very powerful alternative in the current scenario of flaring up antibiotic resistant microbes. These have gained much interest of worldwide researchers for exploration as an inexpensive non-antibiotic alternative approach for the treatment and prevention of a wide variety of infectious diseases.

The IgY therapeutic applications are highly encouraging and further work would pave into the market the entry of novel commercial nutraceuticals or health supplements based on the utility of mono-specific or mixed IgY formulations. Consumer preferences for natural resources to lessen health issues, medicinal costs and the recent move ahead in drug delivery systems and combinational therapeutic regimens it is likely the usage and applications of IgY may swing from functional food category towards development of novel pharmaceuticals in the near future. However, some controversy with regards to the stability of IgY in the GI tract by discovering an effectual way to protect such antibodies from degradation during usage would pave considerable advances in practical directions for optimum utilization of IgY technology and its numerous nutraceutical and therapeutic applications. Though applications of IgY antibodies in fish and aquatic animals have shown a new path for managing the emerging problems posed through infectious pathogens, strengthening of research activities are suggested to see the virtual benefits of EYA in fish and aquatic industry. Additional and future studies can be directed to mode of action of EYA in fish, residue of the antibodies in the body, determination of appropriate dose and suitable formulations in the fish diet and probably side effects of EYA for aquatic animals. There is also need to compare use of IgY antibodies along with other immunostimulant drugs for enhancing effectiveness, feasibility and productivity aspects. Furthermore, commercialization and market attitude should be considered seriously in propagation and consumption of mentioned products in the future.

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