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Review Article

Egg-Derived Antibodies: An Effective Tool in Combating Anti-Microbial Resistance

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Abstract

Egg-derived antibodies' (IgY) preparation against a specific pathogen is an emerging technology for passive immunization. It is an efficient tool which can help to control the global problem of anti-microbial resistance. It is an effective therapeutic alternative having several advantages such as: non-invasive, cost-effective, large amount of IgY production at a single time, also IgY do not react with mammalian antibodies or rheumatoid factors, etc. The main principle behind IgY production is separation of the lipid component of the yolk from its water-soluble fraction. There are various methods described for the extraction of IgY from the egg-yolk like: Water dilution, Poly-ethylene glycol, Dextran sulphate, Ammonium sulphate, Xanthan sulphate, etc. These egg-derived antibodies find application in various fields like: immunodiagnostics, therapeutics, prophylactic, feed additive, etc. This review focuses on the production, importance, uses and applications of egg-derived antibodies.

Keywords: AMR; Egg-Derived Antibodies; IgY; Passive Immunization

Introduction

Anti-microbial resistance (AMR) is emerging as a significant public health issue worldwide. This has contributed to prolonged illness and deaths owing to ineffective treatment. Combating AMR requires multi-faceted one-health approach, involving strict policy interventions, usage of antimicrobial alternatives, proper surveillance of resistance patterns, international cooperation and public awareness [1]. A new antibiotic follows sigmoidal path, it is highly effective and achieves success in its initial lag phase but with the passage of time there is an exponential increase in the resistance against reaching to plateau followed by an equilibrium [2]. Rational use of antibiotics is the most effective way to limit the alarming situation of growing AMR. AMR more often leads to colossal economic losses in three main context- patient perspective (lost earnings of the individual, high rate of morbidity and mortality, drug toxicity), healthcare perspective (money spent on different healthcare programmes, cost incurred on diagnosis and treatment of resistant infections, longer duration of hospital admissions) and societal perspective (productivity losses, impact on trade and tourism due to emerging resistant infections, need for search and development of alternative therapies). Alone in United States, an expenditure of around 2.2 billion USD was estimated on national health care [3]. Irrational use of antimicrobial has led to the development of resistance which is troublesome for their future. The widespread use of antimicrobials as a feed additive in animal feed is one of the major causes of AMR. The bulk in reservoir of antibiotic resistance genes have increased due to extensive use of antibiotics. It has also been documented, that excessive use of antibiotics and the use of sanitizers in the COVID period can enhance this rising problem of AMR especially in the low and the middle-income countries [4]. This increase in the rate of bacterial resistance in the post COVID era has even been warned by WHO [5]. In an antibiotic charged environment a selection pressure builds up favouring the resistant micro-organisms. Moreover, horizontal transfer of the AMR genes

has caused the spread of AMR genes among the microbial population.

Alternatives to antibiotics are effective weapon against AMR, which include plant-based therapies [6], use of phage therapy [7] or generation of therapeutic antibodies [8]. Others involve: probiotics, genetically engineered bacteriophages, innate proteins or bacterial extracts for immune stimulation, vaccines, anti-microbial peptides, nanoparticles, host defense peptides and innate defense peptides and antibiofilm peptides [9]. Among these, therapeutic antibodies are noted to be the fastest growing field in pharmaceutical industry [10]. The extraction of the polyclonal antibodies from the hyperimmunized animal against a certain pathogenic condition is gaining popularity. The extraction of these antibodies from egg yolk of birds is very beneficial due to its specificity and large production. The hens are hyperimmunized for a specific pathogen and then the antibodies are extracted from the egg yolk, which can be further utilized as therapeutic agent against that particular pathogen.

Therapeutic alternatives

- **Plant-based therapy**: Various medicinal herbs and other plant products have effectively been used as therapeutic agents. Moreover, now plant-based vaccines (edible vaccines) are also gaining popularity. Plants and their products are known for their medicinal activity since ancient times [11]. Many plant species such as *Seasamum indicum*, *Cassia tora* and *Anogeissus acuminate* are known for their bactericidal activity. The antibacterial activity of the fluid extracts of *Bergenia ciliata, Jasminium officinale* and *Santalum album* against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa and Escherichia coli* has been documented [12].
- **Phage therapy**: It was in 1919 when bacteriophage therapy was identified by Felix d' Herelle where it was used to treat four patients suffering from bacterial dysentery in Paris [13]. Bacteriophages have a higher penetration capacity at site of infection [9] and hardly cause any adverse reactions in the body when administered as the allergic reactions due to antibiotics. Bacteriophage therapy have been used against enterohaemorrhagic *E. coli* (EHEC) and *Campylobacter* [14]. Phage therapy works on the principle of using the naturally occurring bacteriophages to infect and kill the target bacteria. Recently, the phage-derived lytic proteins are being engineered to prepare chimeras, which have potential antibacterial activity. Phage treatments have also been effectively applied in case of surgical interventions, infected burns, infected ulcers and wound [15]. However, it becomes difficult to identify a therapeutic bacteriophage of a definite specificity. The determination of the dosage of the virus required to be lytic against a particular bacterium is difficult. Also, there is a requirement of a detailed study of the viral genome as it should not contain any antibiotic resistant genes, other bacterial virulence genes or any phage encoded toxin genes.
- **Therapeutic antibodies:** These can be either polyclonal antibodies directly derived from the hyperimmunized serum or egg yolk or monoclonal antibodies against a specific epitope of a pathogen or engineered antibodies targeting a specific pathogen. The great demand for a therapeutic can be fulfilled by the large-scale and economic generation of these polyclonal antibodies. These animal-derived antibodies has been recognized as an effective tool as a therapeutic against various pathogenic infections, venoms and toxins [16,17].
- **Probiotics**: These are the living microorganisms having the antimicrobial activity. Their activity can be attributed to mechanisms such as: changing the microbial flora of the GIT, enhancement of the immune system, production of the antimicrobial compounds and can replace the pathogenic microorganism by competing with them. It has a lower occurrence of adverse reactions. They can be given orally [18] or through enema/nasogastric tubes [19].
- **Genetically engineered bacteriophages:** These are the viruses which have been genetically engineered with some specific improved properties for bacterial lysis. Engineered phages against a specific microbe is a new field. Development of engineered chimeric phages, phages to disrupt the antibiotic resistance genes or plasmids [20] or phage lytic proteins [21].
- **Vaccines:** Vaccines are a part of a prophylactic measure rather than therapeutic. But it can definitely be an alternative to antimicrobials as it prevents the occurrence of disease. Inactivated bacteria or bacterial toxoids or genetically engineered vaccines or recombinant proteins can serve as the vaccine component against a specific pathogen. Proper and timely vaccination helps to prevent most of the diseases and hence no requirement of using the antimicrobials. Vaccines help in the reduction of usage of antibiotics by reducing the occurrence of the bacterial infections that require antibiotic treatment, decreasing the number of infections caused by drug-resistant microorganisms and also the reduction of viral infections for which antibiotics are administered unnecessarily [22].
- **Anti-microbial peptides:** These are small proteins and are capable of direct antimicrobial activity. They may cause bacterial lysis by damaging its cell membrane, forming pores and even destructing bacterial plasmid, RNA and proteins [23]. They are considered as efficient candidates for clinical cases

since they bear the natural property of antimicrobial activity, act speedily and efficiently and has very less chances to develop resistance.

- **Nanoparticles:** These nano-sized particles have been proved to have an anti-bacterial activity which is dependent on their concentration, stability and the temperature of their use [11]. They interact with the bacterial proteins and interfere with the normal bacterial metabolic processes. Anti-bacterial activity of nanoparticles such as: silver nanoparticles, ZnO nanoparticles, copper-oxide nanoparticles and gold nanoparticles, have been established.
- **Anti-biofilm peptides:** These are the new anti-bacterial peptides that prevent the formation of a biofilm of a specific bacterium through nucleotide- signaling molecules. Biofilms show massive tolerance against the antimicrobial agents. First peptide observed to have the anti-biofilm activity was human cathelicidin LL-37 [24]. LL-37 analogue has also shown to inhibit the biofilm of MDR *Staphylococcus aureus* [25].

Passive immunization therapy

Passive immunization is a technique wherein pre-formed antibodies are administered to combat the attacking pathogen. This is beneficial when an immediate action is required against the pathogen. The emergence of drug–resistant microorganisms have popularized the technique of passive immunization. Passive immunization can be given by different ways such as by maternal antibodies during birth, through colostrum (natural immunization) and by administering the specific antibodies (acquired). These antibodies can be hyperimmune polyclonal immunoglobulins, monoclonal antibodies or recombinant (or genetically engineered) antibodies [26].

- **Hyperimmune polyclonal antibodies**: They have been used since long as a therapeutic against microorganisms and venoms. These can be derived from the serum of hyperimmunized individual or from the egg yolk of hyperimmunized hens. Egg yolk is a better option due to the isolation of one isotype (IgY) which is specific and do not show cross reactivity with the mammalian antibodies, however, hyperimmunized serum can show cross-reactivity or induce anaphylaxis.
- **Monoclonal antibodies**: Hybridoma technology by Kohler and Milstein was developed in 1975 to produce specific monoclonal antibodies. These are the antibodies which are produced to target a specific epitope of an antigen and can be utilized effectively as a diagnostic or a therapeutic agent [27].
- **Genetically engineered or recombinant antibodies**: Recombinant of any monoclonal antibody can be obtained by cloning it in an expression vector. Chimeric, humanized and human antibodies have been produced. New generation antibodies include F_c engineered antibodies, antibody fragments, bispecific antibodies, antibodies fused with the effector proteins and intrabodies [28].

Egg yolk and IgY

Egg yolk accounts for around 27.5% of the total hen's eggs. Egg yolk have been shown to have various properties such as antibacterial, antiviral, anticancer, antioxidant, anti-inflammatory, and immunological [29]. It is composed of one-third of proteins and two-third of lipids. Egg components viz., egg yolk antibody (IgY), lysozyme and ovotransferrin are considered as anti-microbial agents. It has also been reported that egg albumen contains ovomucin and ovoinhibitor which has anti-viral properties [30]. IgY is functionally equivalent to mammalian IgG. IgY is major fraction of γ-livetin proteins of plasma proteins of the egg yolk [31]. Birds deposit their maternal antibodies in the egg, out of which IgY is predominantly present in egg yolk, while IgA and IgM are present in egg white. Concentration of IgY in egg yolk ranges from 5-25 mg/ml and that of IgA and IgM in egg albumen is 0.7 mg/ml and 0.15 mg/ml respectively [32].

The production of IgY in the hen's egg is of utmost importance and is recognized allover due to its ability of being utilized for diagnostic, therapeutic, scientific and prophylactic purposes. The amount of immunoglobulins obtained from hen's eggs is around 300 times of that obtained from rabbit blood. It has been documented that around 100-200 mg IgY can be obtained from a single egg, out of which 2-10% is specific for the antigen [33]. The nomenclature of IgY is so because of its predominance in the egg yolk. The genetic sequence of IgY shows much resemblance with human IgE.

Importance of IgY

IgY isolated from hen's egg offers many advantages over IgG isolated from the serum

- Amount of antibodies produced from one egg yolk is equivalent to that from 200-300 ml of mammalian blood.
- Egg yolk IgY do not show any reaction with mammalian antibodies or rheumatoid factors
- They also do not react with bacterial F_c receptors.
- Since they do not activate complement components, there are almost no chances of false positive results in immunoassays [34]
- Collection of IgY from egg yolk is a non-invasive method of obtaining the antibodies unlike the invasive method of bleeding the animal for serum collection.

- Large amount of specific antibodies can be obtained at a single time
- The IgY isolation procedure is also cost-effective.
- Large amount of IgY can be harvested at a single time
- IgY production is also ethically approved as the animal is not undergoing any type of stress like bleeding
- Due to the evolutionary distance between the birds and mammals, IgY recognizes more epitopes of the mammalian antigen, resulting in the amplification of signals [35]
- IgY does not combine with protein A and G (IgG binding proteins)

Transport of IgY to egg yolk

The transfer of antibodies from serum to egg was first observed by Klemperer [36]. IgY from maternal circulation to embryonic circulation is specifically transferred to the egg yolk due to the presence of a highly specific receptor on the yolk membrane surface. There is usually a difference of 5-6 days between the presence of IgY in serum and its appearance in the egg yolk. This transport mainly occurs in two steps: firstly, the receptors present on the ovarian follicles helps to take up the IgY into the egg yolk from hen's blood. Secondly, the transfer of the IgY from the egg yolk to the offspring occurs through the process of embryonic circulation [37,38]. The amount of IgY in the egg yolk following its transport is directly proportional to the amount of serum IgY. It has been observed that around 27-30% of the maternal serum's IgY is transferred to egg yolk [37]. Genotype of the hen also has a major impact on the total presence of IgY in the egg yolk. It has also been demonstrated that the intact F_c portion and the hinge region of IgY play a major role in the transport of IgY from serum to egg yolk.

IgY Extraction from egg yolk

IgY extraction is based on the process of delipidation technique initially to obtain the insoluble lipoproteins and lipids as the presence of lipid tend to interfere with the antibody purification procedure [39]. The lipids are present in large quantity in the egg yolk and the removal of these lipids is a very crucial and necessary step in IgY extraction. The plasma proteins of the yolk have to be separated from the granules and the lipids [40].

Mechanism of IgY action

The mechanism behind the action of egg yolk antibodies simply follows the destruction of the antigenic component following the antigen-antibody interaction. The IgY antibody specific for a pathogen bind to its specific antigenic site and impairs the functioning of the antigen [41]. IgY may even lead to neutralization of venoms and toxins [42], enzymatic activity inhibition [41], causing bacterial lysis by agglutination of the bacteria and inhibition of bacterial attachment and colonization in the gut epithelium [43]. IgY can bind to any of the bacterial antigenic component for its destruction such as flagella, lipopolysachharide, fimbriae or outer membrane proteins.

The specific pathogen or antigen is administered to hen, eggs are collected, yolk is separated, immunoglobulins are isolated which can further be utilized as a diagnostic or a therapeutic. The egg yolk after lyophilization can even be used as a feed additive.

Extraction of IgY

Hyperimmunization of the hen with the specific antigen or whole pathogen, produces the antibody in the hen, which then gets accumulated in the egg yolk after being transferred from the serum. Factors responsible for effective immunization include; dosage and type of antigen used, immunization frequency, adjuvant used, mode of administration of the antigen, interval between immunization and the breed, egg laying capacity or age of the hen used [44].

The extraction of IgY mainly involves the separation of the lipids from the water soluble fraction of the egg yolk (crude immunoglobulin along with some lipoproteins and livetin proteins). The method that can be applied for IgY isolation is dependent on factors like: amount of antibodies required, technology used, quality of the product desired, cost that can be incurred, labour skills and waste management preparations. The separation of the lipid component from the protein component can be done through various means

- By using water dilution method in acidic environment
- By using polyethylene glycol
- By using natural gums- polyanionic polysaccharides
- By giving acidic treatment
- By using combination of solvents- like chloroform and acetone which solubilize the lipids

It has been observed that pH plays key role in IgY isolation. At pH 5.1, 94.5% of IgY was obtained from the water-soluble fraction [45]. Various processes of IgY extraction can be divided into two: precipitation methods and chromatographic methods. There are various methods of IgY isolation: Water dilution method, Polyethylene glycol method, Dextran Sulphate method, Ammonium sulphate method, Xanthan sulphate method. Water dilution method and Xanthan sulphate methods were easy to conduct. Centrifugation process involves the major step in IgY isolation [40]. Water dilution method is used widely for large scale production of IgY antibodies and utilized for industrial use. It is also considered to be the

most cost-effective process. The pH and the extent of dilution of the egg yolk are significant in this method [40]. Use of natural gums for IgY extraction was done by Hatta., *et al*. [46]. They used 12 different gums, out of which λ-carragenan and xanthan were found to be the most effective ones. The use of dextran sulphate along with sodium sulphate and calcium chloride have also been used. Calcium chloride is added so that excess of dextran sulphate gets precipitated. This method also gave good IgY yield ranging from 7.5-15 mg of IgY/ml of egg yolk [47]. Polyethylene glycol use for IgY isolation is also much in use [48]. As per Akita and Nakai [40], water dilution method gave the highest yield of IgY, followed by dextran sulphate, xanthan sulfate and lastly polyethylene glycol method. In a study, dextran sulphate and calcium chloride or phosphotungstic acid and magnesium chloride gave the highest IgY yield of 15.6 and 15.1mg of IgY per ml of egg yolk, respectively with more than 60% purity [49]. The applicability of anionic polysaccharides was judged at different pH and concentrations [50].

Further purification of IgY can be done by various chromatographic techniques: such as anion exchange chromatography, affinity chromatography, thiophilic chromatography, ion exchange chromatography, gel permeation chromatography. Some filteration methods can also be used for IgY purification- funnel filteration, column filteration or ultra-filteration. These filteration methods especially column and ultra- filteration have large applicability for industrial production. Various commercial kits are also available for IgY isolation like Eggcellent (Thermo Pierce), EGGstract (Promega) [39], gammaYolk (Pharmacia Biotech), IMSORB Anti-IgY (Biotez), Kaptiv-GY TM (Technogen) [51].

Uses of IgY

- **IgY as an immunodiagnostic**: IgY is a beneficial tool of immunodiagnosis where the interference with the mammalian IgG antibody is a problem. IgY can be utilized in various immunoassays for verifying the concentration of the antigen by ELISA, RIA immunohistochemistry and other assays.
- **IgY for checking the food quality**: IgY can also be utilized to check the presence of any type of contamination in the food. It can be used to detect the presence of toxic substance or a drug in the food item.
- **IgY as a therapeutic:** Egg yolk powder or the isolated egg yolk antibodies against a specific antigen act as a very effective and an economical therapeutic against that particular antigen. It also helps to reduce the development of antibiotic resistance as it lowers the antibiotic usage.
- **IgY as a prophylactic:** The prophylactic functions of IgY have also been investigated by many scientists. IgY against *Pseudomonas aeruginosa* have been tested by giving it orally in cystic fibrosis patients, which significantly decreased the incidence of the disease.
- **IgY as a feed additive:** Whole egg powder or the egg yolk powder containing the antibodies for a specific pathogen can be used as an additive in poultry or swine feed.
- **IgY in immunochromatography:** Immunoaffinity chromatography is the method of purification of certain molecule from a complex of molecules by the principle of the highly specific antigen-antibody reactions. Immobilized egg yolk antibodies have been used for the isolation of a particular protein. Moreover, IgY use in generation of this technique is limited due to its high cost and decreased efficiency of immobilization of the antibody.
- **Applications of IgY:** The concept of production of egg yolk antibodies have been applied in various fields and for many purposes [52]. There are also many commercial preparations based on this (Table 1- 3) [53].

Characteristics	Polyclonal antibody	Monoclonal antibody
Time consumption	Requires less time after the first immunization (few months)	Can take upto a year or even longer
Cost of production	Less expensive	More expensive
Requirement of technical expertise	Comparatively less	More
Specificity	It is heterogenous- recognize number of epitopes	It is monospecific
Stability	More stable	Less stable. Highly susceptible to changes in pH and salt concentration
Concentration and purity	Comparatively lower	Higher

Table 1: Difference between polyclonal and monoclonal antibody.

Characteristics	IgY	Igg
Molecular weight	180 kDa	159 kDa
Heavy chain	Larger molecular weight: 68 kDa	50 kDa
Heavy chain domains	5 domains- 1 variable and 4 constant domains	4 domains- 1 variable and 3 constant domains
Hinge region	Absent	Present between Cγ1 and Cγ2 domain
Flexibility	Less flexible than IgG	More flexible

Cystein residues	Has 2 additional cysteine residues- Cys 331 and Cys 338	Absent
Stability	Less stable to acid denaturation and heating (stable between 60-70° C)	Comparatively more stable
Isoelectric point	Lower than IgG (5.7-7.6)	Higher than IgY
pH stability	Gets inactivated at below pH = 3	Activity persists even at pH = 2
Proteolysis stability	More susceptible to trypsin, chymotrypsin and pepsin	Less susceptible
Antibody extraction process	Non-invasive- by collection of eggs	Invasive- by bleeding and collecting serum

Table 2: Comparison between IgY and IgG.

Product name	Company name	Targeted action
Ovopron IgY	Pharma Foods International Co. Ltd	Prophylactic and therapeutic action against *H. pylori* infection in humans
i26	Arkion Life Sciences	Act against 26 enteric pathogens in humans
IgY Recovery Proteins	Arkion Life Sciences	Reduce muscle soreness and improve overall performance in athletes
i26 Companion	Arkion Life Sciences	improve immune function in cats and dogs
Protimax	Arkion Life Sciences	feed supplement against bovine and porcine enteric pathogens
Globigen	EW Nutrition, along with Ghen Corporation	IgY feed supplement for animals
Ovalgen	EW Nutrition, along with Ghen Corporation	IgY feed supplement for humans
Ig-Guard	AD Biotech Co. Ltd.	Act against GI pathogens in animals and viruses in aquatic animals
Ig-Lock	Dan Biotech Inc.	Act against GI pathogens in animals and viruses in aquatic animals
BIG	Aova Technologies	Egg yolk antibody (IgY) against the enzyme PLA2

Table 3: Commercial preparations of egg yolk antibodies [53].

Future prospects

There still remains a lot of scope of research and development in this technology of egg yolk antibodies production. Egg yolk antibodies should be generated against more diseases especially the most prevalent ones and those which show increased anti-microbial resistance. More research should be conducted for the choice of the adjuvant to be given along with the antigen for the generation of a better immune response. Furthermore, research should be conducted to generate the egg-yolk antibodies against multiple pathogens to act against several diseases at the same time. Combination product of IgY along with some other therapeutic alternative should be researched upon and produced.

Conclusion

Egg-yolk antibodies' production against a specific antigen is very productive and a cost-effective technology and can be applied to various fields of therapeutics, diagnostics, prophylactics, etc. It is a very good non-invasive alternative for mammalian immunoglobulins. These methods of passive immunization are gaining fame and are being demanded because of the global prevalence of antibiotic resistant pathogens. IgY technology also comes out to be an ideal choice for the production of antigen specific antibodies in large amounts. Due to several advantages, it has gained the interests of many scientists, though it still has a lot of scope of many modifications, improvements and advancements.

Conflict of Interest

The authors have disclosed no potential conflicts of interests.

Bibliography

1. Carbon C., *et al*. "Moving from recommendation to implementation and audit: Part I, Current recommendations and programsL: a critical commentary". *Clinical Microbiology and Infection* 8 (2012): 92-106.

2. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India". *Journal of Biosciences* 33 (2008): 593-603.

3. Thorpe KE., *et al*. "Antibiotic-resistant infection treatment costs have doubled since 2002 now exceeding $2 billion annually". *Health Affairs* 37 (2018): 662-669.

4. Bandyopadhyay S and Samanta I. "Antimicrobial Resistance in Agri-Food Chain and Companion Animals as a Re-emerging Menace in Post-COVID Epoch: Low-and Middle-Income Countries Perspective and Mitigation Strategies". *Frontiers in Veterinary Science* 7 (2020): 620.

5. World Health Organization (WHO) (2020).

6. Tan BKH and Vanitha J. "Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: A review". *Current Medicinal Chemistry* 11 (2004): 1423-1430.

7. Tiwari R., *et al*. "Bacteriophage therapy: A novel tool for combating bacterial diseases of poultry-A review". *Journal of Immunology and Immunopathology* 13 (2011): 55-66.

8. Yegani M and Korver DR. "Application of egg yolk antibodies as replacement for antibiotics in poultry". *World Poultry* 23 (2007): 22-25.

9. Owens B. "A ray of hope amid antibiotic gloom". *The Pharmaceutical Journal* 297 (2007): 7893.

10. Chala G., *et al*. "A Review on: Antibody Engineering for Development of Therapeutic Antibodies". *International Journal of Immunology 3*.3 (2015): 27-36.

11. Suay-García B and Pérez-Gracia MT. "The Antimicrobial Therapy of the Future: Combating Resistances". *Journal of Infectious Diseases and Therapy* 2 (2014): 146.

12. Khan UA., *et al*. "Antibacterial activity of some medicinal plants against selected human pathogenic bacteria". *European Journal of Microbiology and Immunology* 3.4 (2013): 272-274.

13. Chanishvili N. "Phage therapy--history from Twort and d'Herelle through Soviet experience to current approaches". *Advances in Virus Research* 83 (2012): 3-40.

14. Carillo CL., *et al*. "Bacteriophage therapy to reduce Campylobacter Jejuni colonization of Broiler chickens". *Applied and Environmental Microbiology* 71 (2005): 6554-6563.

15. Morozova VV., *et al*. "Applications of Bacteriophages in the Treatment of Localized Infections in Humans". *Frontiers in Microbiology* 9 (2018): 1696.

16. Eddleston M and Persson H. "Acute plant poisoning and antitoxin antibodies". *Journal of Toxicology: Clinical Toxicology* 41.3 (2003): 309-315.

17. Han S., *et al*. "Effect of dietary supplementation with hyperimmunized hen egg yolk powder on diarrhoea incidence and intestinal health of weaned pigs". *Food and Agricultural Immunology* 30.1 (2019): 333-348.

18. Szajewska H., *et al*. "Meta-analysis: Lactobacillus reuteri strain DSM 17938 (and the original strain ATCC 55730) for treating acute gastroenteritis in children". *Beneficial Microbes* (2014).

19. Grehan MJ., *et al*. "Durable alteration of the colonic microbiota by the administration of donor fecal flora". *Journal of Clinical Gastroenterology* 44.8 (2010): 551-561.

20. Yosef I., *et al*. "Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria". *Proceedings of the National Academy of Sciences of the United States of America* 112 (2015): 7267-7272.

21. Hodyra-Stefaniak K., *et al*. "Mammalian Host- Versus-Phage immune response determines phage fate *in vivo*". *Scientific Reports* 5 (2015): 1480.

22. O' Neill J. "Vaccines and alternative approaches: Reducing our dependence on antimicrobials". *The Review on Antimicrobial Resistance* (2016).

23. Peters BM., *et al*. "Antimicrobial peptides: primeval molecules or future drugs?" *PLOS Pathogens* 6 (2010): e1001067.

24. Jacobsen AS and Jenssen H. "Human cathelicidin LL-37 prevents bacterial biofilm formation". *Future Medicinal Chemistry* 4 (2012): 1587-1599.

25. Haisma EM., *et al*. "LL-37- derived peptides eradicate multidrug-resistant Staphylococcus aureus from thermally wounded human skin equivalents". *Antimicrobial Agents and Chemotherapy* 58 (2014): 4411-4419.

26. Hauram JS. "Recombinant polyclonal antibodies: the next generation of antibody therapeutics?" *Drug Discovery Today* 11 (2006): 13/14.

27. Maynard JA., *et al*. "Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity". *Nature Biotechnology* 20 (2002): 597-601.

28. Chames P., *et al*. "Therapeutic antibodies: successes, limitations and hopes for the future". *British Journal of Pharmacology* 157 (2009): 220-233.

29. Mine Y. "Egg bioscience and biotechnology". Wiley-Interscience, Hoboken, NJ (2008).

30. Watanabe K., *et al*. "Binding activities of pronase-treated fragments from egg white ovomucin with anti-ovomucin antibodies and Newcastle disease virus". *Journal of Agricultural and Food Chemistry* 46 (1998): 4501-4506.

31. Kovacs-Nolan J., *et al*. "Advances in the value of eggs and egg components for human health". *Journal of Agricultural and Food Chemistry* 53 (2005): 8421-8431.

32. Li X., *et al*. "Effects of egg and yolk weights on yolk antibody production in laying chickens". *Poultry Science* 77 (1997): 266-270.

33. Munhoz LS., *et al*. "Avian IgY antibodies: characteristics and applications in immunodiagnostic". *Ciência Rural, Santa Maria* 44.1 (2014)153-160.

34. Carlander DH., *et al*. "Peroral immunotherapy with yolk antibodies for the prevention on and treatment of enteric infections". *Immunologic Research* 21 (2000): 1-6.

35. Spillner E., *et al*. "Avian IgY antibodies and their recombinant equivalents in research, diagnostics and therapy". *Biologicals* 40.5 (2012): 313-322.

36. Klemperer F. "Ueber natürliche immunität und ihre verwerthung für die immunisirungstherapie". *Archiv **für** die Experimentelle Pathologie und Pharmakologie* 31 (1893): 356-382.

37. Hamal KR., *et al*. "Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens". *Poultry Science* 85 (2006): 1364-1372.

38. Gregory W. "IgY: Clues to the origins of modern antibodies". *Immunology Today* 16 (1995): 392-398.

39. Tan SH., *et al*. "A novel, cost-effective and efficient chicken egg IgY purification procedure". *Journal of Immunological Methods* 380 (2012): 73-76.

40. Akita EM and Nakai S. "Production and purification of Fab' fragments from chicken egg yolk immunoglobulin Y (IgY)". *Journal of Immunological Methods* 162 (1993): 155.

41. Rahman S., *et al*. "Oral passive IgY-based immunotherapeutics: a novel solution for prevention and treatment of alimentary tract diseases". *Human Vaccines and Immunotherapeutics* 9 (2013): 1039-1048.

42. Wang LH., *et al*. "Characterization of chicken egg yolk immunoglobulins (IgYs) specific for the most prevalent capsular serotypes of mastitis causing Staphylococcus aureus". *Veterinary Microbiology* 149 (2011): 415-421.

43. Jin LZ., *et al*. "*In vitro* inhibition of adhesion of enterotoxigenic Escherichia coli K88 to piglet intestinal mucus by egg-yolk antibodies". *FEMS Immunology and Medical Microbiology* 21 (1998): 313-321.

44. Li X., *et al*. "Chicken egg yolk antibodies (IgY) as non-antibiotic production enhancers for use in swine production: a review". *Journal of Animal Science and Biotechnology* 6 (2015): 40.

45. Akita EM and Nakai S. "Immunoglobulins from egg yolk: isolation and purification". *Journal of Food Science* 57 (1992): 629-633.

46. Hatta H., *et al*. "A novel isolation method for hen egg yolk antibody, "IgY". *Agriculture and Biological Chemistry* 54 (1990): 2531-2535.

47. Jensenius JC., *et al*. "Eggs: conveniently packaged antibodies. Methods for purification of yolk IgG". *Journal of Immunological Methods* 46 (1981): 63-68.

48. Polson A., *et al*. "Improvements in the isolation of IgY from the yolks of eggs laid by immunized hens". *Immunological Investigations* 14 (1985): 323-327.

49. Deignan T., *et al*. "Comparative Analysis of Methods of Purification of Egg Yolk Immunoglobulin". *Food and Agricultural Immunology* 12.1 (2000): 77-85.

50. Chang H., *et al*. "Isolation of immunoglobulin from egg yolk by anionic polysaccharides". *Journal of Agricultural and Food Chemistry* 48 (2000): 995- 999.

51. Staak C., *et al*. "Isolation of IgY from Yolk. Chicken Egg Yolk Antibodies, Production and Application". Springer-Verlag Berlin Heidelberg (2001).

52. Pereira E., *et al*. "Egg yolk antibodies (IgY) and their applications in human and veterinary health: A review". *International Immunopharmacology* 73 (2019): 293-303.

53. Kovacs-Nolan J and Mine Y. "Egg Yolk Antibodies for Passive Immunity". *Annual Review of Food Science and Technology* 3 (2010): 163-182.