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Avian Antibodies (IgY) to Fight Antibiotic Resistance

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Introduction

The alarming increase of resistant microbes (bacteria, viruses, fungi, protozoa) is today one of the biggest threats to both mankind and environment. This article will push for the use of avian antibodies as a replacement or complement to antibiotics and thereby diminish the development of antibiotic resistant microbes.

Antibiotic resistance and how to fight it

The big load of antimicrobials (antibiotics, antimycotics and antivirals) given both to man and animals is a threat to our environment with the menace to give a total imbalance between microbial species in humans, animals, plants, water and soil.

Antibiotics are today found ubiquitous in our environment in constantly growing amounts. Antibiotics are given to animals as "growth factors"-especially to piglets, calves and chickens-to avoid weaning diarrhoea and to promote growth [1]. It is forbidden in Europe since 2006 and also in some states in USA. But they are still used widely in other countries. Antibiotics are distributed everywhere in animals and humans-blood, flesh, urine, faeces. Antibiotic contaminated flesh is eaten both by humans and animals. Antibiotics from animals and humans are excreted to the environment by urine and faeces. Antibiotic factories spread out tons of antibiotics from their plants. Unused antibiotics are thrown away. Soil and water are enormous reservoirs for antibiotics. Soil-dwelling microbes encounter a myriad of antibiotics mostly in low concentrations. Low concentrations do not kill bacteria, but they will evolve strategies to resistance. Multi resistant bacteria are found in excrements of birds far out in the wilderness of Greenland. Genetic spread of multidrug resistant bacteria is the biggest problem for propagation of antibiotic resistance. The gastrointestinal canal of humans and animals are "mating" places for bacteria to exchange their genetic material. Hospitals-especially intensive care unit (ICU) and new-born wards-are important domains for spread of genetic resistance. Cross-infections are the major impediment for the control of resistance.

European Union has decided to give a strong support for depression of resistant bacteria by formulating guidelines to control use of antibiotics (European Union Official Journal, nr C, 14.11.2013). The Infectious Diseases Society of America (Bad bugs, no drugs: Public Health Crisis Brews, 2004) has proposed legislative and federal solutions to this emerging health problem: Well controlled hygiene, isolation of patients with severe infections, careful antibiotic prescribing.

The recommended measures are not enough! For an optimal antibiotic control we need to think in new directions or rather go back to old ideas-according to Rousseau: "Retournons a la nature"!

The best defence for humans, mammals and birds against all kind of infectious agents has always been the immune system. A powerful way to fight infections in our days will be to use this system as complement or replacement to antibiotics.

Antibodies

Antibodies have a high specificity for binding and inactivating foreign substances including different microbes (bacteria, viruses, fungi, protozoa). The immune system and microorganisms have coexisted for millions of years and microorganisms have not become resistant towards them.

All mammals produce antibodies. Lately, much interest has been turned to antibodies from eggs of hens (avian antibodies, IgY). The yield of antibodies from eggs is much larger than can be achieved from any mammal. The collection of antibodies from egg yolk does not comprise any bleeding. European centre for the validation of alternative methods (ECVAM) strongly recommends avian antibodies as alternative to mammalian antibodies.

Antibodies from egg yolk (IgY)

Antigen specific avian antibodies (IgY) have been used for numerous applications in medical and research fields. One of the most valuable and promising areas of IgY is its potential to be used for passive immunization to treat and prevent human and animal infections. Much research has been done both *in vitro* and on animals and humans. However, clinical applications of IgY in humans are very scarce. For references see below: "IgY in therapies".

Avian IgY is the functional equivalent to mammalian IgG and is the predominant immunoglobulin in egg yolk [2]. IgY is actively transferred from the serum of the hen into the egg yolk to give immunity to embryos and chickens. This corresponds to placental IgG transfer in mammals to provide immunity to the fetus. The overall structure of avian IgY is similar to mammalian IgG (Figure 1), with two heavy and two light chains, but there are some important differences. The molecular mass of mammalian IgG is 150 kDa, whereas it is approximately 180 kDa for avian IgY. The greater molecular mass is due to increased number of heavy chain constant domains and a pair of extra carbohydrate chains. The hinge region of IgY is shorter and less flexible.

The evolutionary spread means that there is no immunological cross reactivity between avian IgY and mammalian IgG. Avian antibodies do not activate the complement system.

Antibodies are absorbed from the intestine in young piglets and calves during the first 24 hours to 48 hours post natum [3]. After this time period there will be no absorption of active antibodies from the gastrointestinal tract in animals. All studies in humans, including

infants, show, that there is no absorption from the intestine [4], but studies on new-borns are scares or not done. Since IgY is not absorbed from the intestinal canal, there will not be any reactions outside the canal and no risk for toxicity.

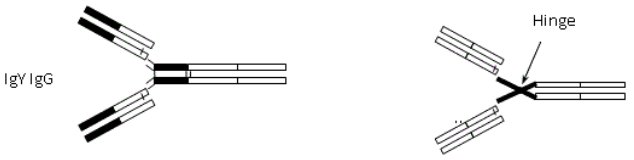

Figure 1: Imunglobulin Y and Immunoglobulin G.

A hen usually lays ~280 eggs in a year and an egg yolk contains 100–150 mg of IgY antibodies. This results in a yield of 28–42 g IgY per year per hen [5]. When a hen is vaccinated against a microbe, around 5%-10% of the IgY will react to this microbe. The production is simple, efficient and economical. It involves separation of the egg yolk from the white, followed by purification of antibodies from lipids and other materials. Avain IgY for therapeutic use can be produced from the egg yolk without any other ingredients than sterile water. IgY is stable at pH 4-9 and up to 65°C in aqueous condition. High salt conditions and stabilizing regents (e.g. sucker) increases the stability to heat, acid pH and high pressure.

IgY fractions have been stored in at +4°C for 20 years without any significant loss of antibody titer. The yield can easily be scaled up to produce enormous quantities of specific protective antibodies. Eggs are an essential part of the normal diet of man. There is practically no risk for side effects when taken orally. However, it is important to consider egg allergy before starting therapy with egg yolk antibodies [5].

IgY in therapy

There are thousands articles about the use of IgY both *in vitro* and animal studies, but there are very few human studies.

Bacteria

Anti-pseudomonas IgY and Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in a gene seven, encoding the cystic fibrosis transmembrane conductance regulator [6]. This genetic defect leads to abnormally thick mucus [7] and predisposes to respiratory infections, which are the major cause of morbidity and mortality in CF. Several bacteria may cause respiratory infections in CF, but chronic *Pseudomonas aeruginosa* (PA) infections ultimately occur in virtually all of them. Once a chronic PA-infection has been established, eradication is hardly ever possible, even with high doses of antibiotics. Patients with CF are a group of patients, who has the highest incidence of multi-resistant bacteria in the world.

Anti-pseudomonas IgY (IgYPseud) reduces PA adhesion to epithelia. Flagellin is the major antigen to which IgYPseud binds. This binding prevents PA [8] to invade the host.

Flagellin is the main protein of flagellae and is crucial for establishing infections in hosts. Flagellin effects PA chemotaxis, motility, adhesion and inflammation. In an *in vitro* test with PMN cells there was a statistically significant ($p<0.05$) higher bacterial killing with IgYPseud compared to non-IgY controls. This protective activity may be explained by an opsonising effect of IgYPseud to *Pseudomonas* [9] Vaccination with other bacteria that have flagellae, will probably give similar immunologic reaction, as we have found for PA. "Antiflagellin"-IgY has potential to be effective for treatment against PA and probably also for other bacteria with flagellae.

The first big clinical study in humans is our on patients with CF [10,11]. CF-patients have been gargling every night with IgYPseud and thereafter swallowing the drug. IgYPseud prevents colonization of PA (statistically significant, $p=0.011$) and reduces the number of antibiotic treatments. Anti-Pseud IgY was approved to be sold on license to name-given CF patients in Sweden for the prevention of PA infections in 2002 (Clinical trials.gov/NCT00633191) and got Orphan Drug Designation by EU in 2008 (EMEA/COMP/325516/2008). The study is still going on. Hitherto>100.000 doses have been taken with same good results and without any advert events.

Single trials indicate that IgYPseud might be of help also for infections in other locations. One boy, who had an outer ear infection with PA, which did not respond to antibiotics, was promptly helped by IgYPseud droplets administered in the outer ear (unpubl.). A patient with severe burns was helped by local application of compresses with IgYPseud solution (unpubl.).

Gastro-enteric infections

IgY can easily be administered orally for the treatment of gastrointestinal infections [12]. A fraction of immune globulins retain their neutralization activity in various segments of the gastrointestinal tract. A stronger activity would be easy to accomplish by buffers or in acid resistant capsules (see "*chlostridium difficile*").

Helicobacter pylori: IgY against *H. pylori* has been studi ed together with lanzoprazol in human volunteers with good results [13,14].

Enterotoxic *Escherichia coli* (ETEC): ETEC is a frequent cause of diarrhea for children in developing countries as well as for travelers to these countries. It accounts for around one million deaths yearly. Oral administration of IgY-ETEC is proven to be successful for the treatment of gastrointestinal infections in animals (see: "fodder"). This indicates a good hope that it will be of value also for humans.

Salmonella species: The passive protective efficacy of avian IgY to control experimental salmonellosis in mice [15] and calves [16] has been examined. The animals treated with specific IgY showed increased survival. IgY inhibits the adhesion of *Salmonella enteritidis* to human intestinal cells *in vitro* [17] indicating that it has potential for salmonellosis in humans.

Chlostridium difficile: A test of the passage through the human intestine for bovine IgG for *chlostridium difficile* was done by Chelly et al. They found that stool samples neutralized the cytotoxicity of *c. difficile* toxins A and B. A low dose (1.6%-3.8 %) of given fecal bovine IgG was found in regular stools. The dose was increased to 8.8 % after having given omeprazol, and was still more increased after having given the IgG in enteric capsules (32.7%) [18]. I have not found these experiments for IgY, but they would hopefully give similar results.

Oral infections

Streptococcus mutans causes caries. A mouth rinse containing IgY-mutans reduces the establishment of these bacteria in dental plaques of

humans [19]. The antibodies inhibit *S. mutans* adherence to saliva-coated hydroxyapatite discs *in vitro* and decrease the percentage of *S. mutans* per total streptococci *in vivo* [20]. Chewing gums with anti-strepmutans IgY against caries are sold commercially in Japan.

***Hemolytic streptococcus A* IgY** would be a perfect prophylaxis and treatment for tonsillitis, but I have not seen any such studies.

Gingivitis: Several studies on specific IgY have been studied in mice on bacteria causing gingivitis and halitosis (bad breath) such as *Prevotella intermedia*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* [21]. All results were convincing with less gingival infections, less plaques and less gingival bleeding. For fusobacterium the histopathological slides of the gums were improved after IgY treatment for 15 days.

Pharyngitis: In a double blind study from China, patients were treated with IgY from eggs of hens vaccinated with different non-defined bacteria from pharynx. Either pharynx-specific IgY or placebo was sprayed six times daily into mouth and pharynx. The group treated with specific IgY had significantly less symptoms of pharyngitis than the placebo group ($p<0.01$) [22].

Multiresistant bacteria; ESBL,MRSA and VRE

The emergence of multiresistant bacteria such as MRSA (multiresistant *Staphylococcus aureus*), ESBL(extended-spectrum β-lactamase)-producing bacteria and VRE (vancomycin resistant enterococci) is a serious dilemma. The number of reports on infections with these dreadful bacteria has dramatically increased over the last decades. Multidrug-resistant *E. coli* strains disseminate worldwide for which there are no new effective antibiotics. Potentially a "super bug," resistant to all antibiotics may emerge in the near future. Constant and careful worldwide surveillance for multidrug-resistant bacteria is urgently warranted [23].

Specific IgYs for multiresistant bacteria have potential to be a new effective way to treat them. IgY-ESBL has been produced from hens vaccinated with bacteria carrying ESBL genes and found to have specific binding capacity. Despite the tremendous need to find an effective treatment for ESBL, MRSA and VRE infections, there is very little done for possibilities to treat these bacteria with IgY. Clinical studies on this prospect are urgently needed.

Viruses

Influenza

Influenza viruses remain a major threat to global health due to their ability to undergo changes through antigenic drift and antigenic shift. IgY antibodies against influenza can be administered passively in humans (orally and probably intranasally) and can be used quickly and safely to help in the fight against a pandemic influenza. In a study using inactivated H1N1, H3N2, and H5N1 influenza viruses to immunize hens a high level of anti-influenza virus IgY was induced in sera and eggs, which lasted for at least 2 months after two immunizations [24]. *In vitro* IgY inhibited homologous as well as heterologous strains of viruses. In a mouse model, IgY to H5N1 protected 100% of the mice against lethal challenge with H5N1, when administered intranasally 1 h prior to infection. Of particular interest was the finding that IgY to H5N1 cross-protected against A/Puerto Rico/8/34 H1N1. Based on these animal results, it is very plausible that IgY-Influenza can be used to prevent and control influenza viral infections.

Many countries including Vietnam introduced mass vaccination of poultry with H5N1 vaccines. In a study from Vietnam eggs were bought directly on the supermarket. Specific IgY-H5N1 was found in these eggs. When administered intranasally in mice before and after lethal infection with H5N1 and related H5N2, IgY could prevent infection resulting in complete recovery [25].

***Human rotavirus* (HRV)** is the major contributing agent of acute infantile gastroenteritis, resulting in more than one million deaths annually. HRV causes shortening and atrophy of the villi of the small intestine, followed by decreased water absorption leading to severe diarrhea and vomiting. Oral administration of IgY from eggs from hens immunized with three different serotypes of rotavirus (mouse, human and monkey) prevented diarrhea in mice infected with murine rotavirus [20,25].A small clinical human study in Bangladesh gave promising results, but was never followed up [26].

Fungi

Candida albicans is a tremendous burden for oncologic patients treated with cytotoxic drugs and antibiotics. More or less severe candidiasis in mouth and throat will occur in more than 75% of leukemic patients during the period, when cytotoxic drugs are given intensively. In a study IgY candida was given prophylactly as a mouth-wash every day to four children with leukemia during intensive treatment with cytotoxic drugs. None of them got any candidiasis, but three of four controls [27].

IgY in Fodder and in Fish Cultivations

In fodder

Enteric colibacillosis by ETEC encountered in neonatal calves [28] and piglets [29] is a major cause of death in these animals. The passive protective effect of IgY against induced diarrhea by ETEC has been studied in these animals. In both it gave a good prophylactic and therapeutic result.

In fish-cultivations

Yersinia: IgY against *Y. ruckeri* protects rainbow trout from infection [30].

Edwardsiella: Anti-Edwardsiella IgY prevents Japanese eels from Edwardsiellosis infected with *Edwardsiella tarda* [31] Table 1.

Microbe(s)	*In vitro*	Animals	Humans	Comments	Ref(s)
Pseudomonas	+	+	+	Longest clinical study	[8-11]
ESBL	+			*In vitro* tests: Promising results	-

MRSA	+			Good candidate for IgY treatment.	-
E. coli	+	+	+	In fodder, good results	[12]
EHEC	+	+		Good result in mice	
ETEC	+	+		Good results in piglets and calves	[28,29]
EPEC	+			Infantile diarrhoea, Brazil	
Salmonella	+	+		Good results in animals	[15-17]
Helicobact.p.	+	+		Together with antacidum.	[13,14]
Enterobacter.c			+	Prematurely born infants, nonpubl.	-
Strep.mutans			+	In lozenges (also in chewing gums)	[19,20]
Pharyngitis			+	Unknown bacteria. Good results. Chinese	[22]
Gingivitis	+	+	+	Article in Chinese	[21]
Rotavirus	+	+	+	Many animal studies. At least 1 human study	[26]
Hepatit A, B	+			*In vitro* test: A. Review article B.	[32]
Candida albic.	+	+	+	Oncology, immundepressed pats	[27]
Influenza,H5N1	+	+		AntiH5N1 IgY in eggs from supermarket in Vietnam	[24]
Yersinia		+		Rainbow trout	[30]
Edwardsiella		+		Japanese eels	[31]
Coccoidosis		+		Study on protection of chickens, fodder.	[33]

Table 1: Excerpt of studies on IgY.

Conclusion

An abundance of studies on specific IgY antibodies from immunized hens conclusively suggest that they are very strong weapons against a series of common infections and can be used both as a complement and/or an alternative to antibiotics. Since IgY is not absorbed from the gastro-intestinal canal, it is especially attractive for peroral or local immunotherapy for a variety of bacterial, viral and fungal infections in the gastro-intestinal canal, including mouth, throat and airways, as well as for treatment on the skin and other local infections. In addition, specific IgY can be of tremendous help in fodder and for fish-cultivation.

It is high time for all involved in fighting infectious diseases-politicians, health authorities, pharmaceutical companies, physicians, researchers etc., to join in a strong pull for avian antibodies-IgY.

Use of IgY will diminish the development of antibiotic resistant microbes.

References

1. Aarestrup FM (2000) Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. APMIS Suppl 101: 1-48.
2. Warr GW, Magor KE, Higgins DA (1995) IgY: clues to the origins of modern antibodies. Immunol Today 16: 392-398.
3. Erhard MH, Göbel E, Lewan B, Lösch U, Stangassinger M (1997) [Systemic availability of bovine immunoglobulin G and chicken immunoglobulin Y after feeding colostrum and whole egg powder to newborn calves]. Arch Tierernahr 50: 369-380.
4. Losonsky GA, Johnson JP, Winkelstein JA, Yolken RH (1985) Oral administration of human serum immunoglobulin in immunodeficient patients with viral gastroenteritis. A pharmacokinetic and functional analysis. J Clin Invest 76: 2362-2367.
5. Akita E, Nakai S (1992) Immunoglobulins from egg yolk: Isolation and purification. J Food Science 57: 629-634.
6. Bernhisel-Broadbent J, Yolken RH, Sampson HA (1991) Allergenicity of orally administered immunoglobulin preparations in food-allergic children. Pediatrics 87: 208-214.
7. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, et al. (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245: 1073-1080.
8. Ramsey BW (1996) Management of pulmonary disease in patients with cystic fibrosis. N Engl J Med 335: 179-188.
9. Nilsson E, Amini A, Wretlind B, Larsson A (2007) Pseudomonas aeruginosa infections are prevented in cystic fibrosis patients by avian antibodies binding Pseudomonas aeruginosa flagellin. J Chromatogr B Analyt Technol Biomed Life Sci 856: 75-80.
10. Thomsen K, Christophersen L, Bjarnsholt T, Jensen PO, Moser C, et al. (2012) Anti-pseudomonas IgY antibodies opsonize Pseudomonas aeruginosa augmenting the phogocytosis activity of polymorph nuclear neutrophils. J Cystic fibrosis 11: S38.
11. Kollberg H, Carlander D, Olesen H, Wejåker PE, Johannesson M, et al. (2003) Oral administration of specific yolk antibodies (IgY) may prevent Pseudomonas aeruginosa infections in patients with cystic fibrosis: a phase I feasibility study. Pediatr Pulmonol 35: 433-440.

12. Nilsson E, Larsson A, Olesen HV, Wejåker PE, Kollberg H (2008) Good effect of IgY against Pseudomonas aeruginosa infections in cystic fibrosis patients. Pediatr Pulmonol 43: 892-899.
13. Mine Y, Kovacs-Nolan J (2002) Chicken egg yolk antibodies as therapeutics in enteric infectious disease: a review. J Med Food 5: 159-169.
14. Yang YH, Park D, Yang G, Lee SH, Bae DK, et al. (2012) Anti-Helicobacter pylori effects of IgY from egg york of immunized hens. Lab Anim Res 28: 55-60.
15. Nomura S, Suzuki H, Masaoka T, Kurabayashi K, Ishii H, et al. (2005) Effect of dietary anti-urease immunoglobulin Y on Helicobacter pylori infection in Mongolian gerbils. Helicobacter 10: 43-52.
16. Yokoyama H, Umeda K, Peralta RC, Hashi T, Icatlo FC Jr, et al. (1998) Oral passive immunization against experimental salmonellosis in mice using chicken egg yolk antibodies specific for Salmonella enteritidis and S. typhimurium. Vaccine 16: 388-393.
17. Yokoyama H, Peralta RC, Umeda K, Hashi T, Icatlo FC Jr, et al. (1998) Prevention of fatal salmonellosis in neonatal calves, using orally administered chicken egg yolk Salmonella-specific antibodies. Am J Vet Res 59: 416-420.
18. Kelly CP, Chetham S, Keates S, Bostwick EF, Roush AM, et al. (1997) Survival of anti-Clostridium difficile bovine immunoglobulin concentrate in the human gastrointestinal tract. Antimicrob Agents Chemother 41: 236-241.
19. Nguyen SV, Icatlo FC Jr, Nakano T, Isogai E, Hirose K, et al. (2011) Anti-cell-associated glucosyltransferase immunoglobulin Y suppression of salivary mutans streptococci in healthy young adults. J Am Dent Assoc 142: 943-949.
20. Hatta H, Tsuda K, Ozeki M, Kim M, Yamamoto T, et al. (1997) Passive immunization against dental plaque formation in humans: effect of a mouth rinse containing egg yolk antibodies (IgY) specific to Streptococcus mutans. Caries Res 31: 268-274.
21. Jiang DC, Xu Y, Sun XY, Wang C, Shen JL (2011) [The preparation, identification and physicochemical properties of anti-Porphyromonas gingivalis IgY]. Zhonghua Kou Qiang Yi Xue Za Zhi 46: 586-589.
22. Xie MQ, Meng YX, Li ZH, Li YQ, Zhang KX, et al. (2004) [Effect of specific immunoglobulin Y in the treatment of acute and chronic pharyngitis]. Zhonghua Er Bi Yan Hou Ke Za Zhi 39: 112-115.
23. Chong Y, Ito Y, Kamimura T (2011) Genetic evolution and clinical impact in extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Infect Genet Evol 11: 1499-1504.
24. Wallach MG, Webby RJ, Islam F, Walkden-Brown S, Emmoth E, et al. (2011) Cross-protection of chicken immunoglobulin Y antibodies against H5N1 and H1N1 viruses passively administered in mice. Clin Vaccine Immunol 18: 1083-1090.
25. Yolken RH, Leister F, Wee SB, Miskuff R, Vonderfecht S (1988) Antibodies to rotaviruses in chickens' eggs: a potential source of antiviral immunoglobulins suitable for human consumption. Pediatrics 81: 291-295.
26. Sarker SA, Casswall TH, Juneja LR, Hoq E, Hossain I, et al. (2001) Randomized, Placebo-Controlled, Clinical Trial of Hyperimmunized Chicken Egg Yolk Immunoglobulin in Children With Rotavirus Diarrhea. J Pediatr Gastroenterol Nutr 32: 19-25.
27. Wilhelmsson M, Carlander D, Kreuger A, Kollberg H, Larsson A (2005) Oral treatment with yolk antibodies for the prevention of C. albicans infections in chemotherapy treated children. A feasibility study. Food and agricultural Immunology 16: 41-45.
28. Ikemori Y, Kuroki M, Peralta RC, Yokoyama H, Kodama Y (1992) Protection of neonatal calves against fatal enteric colibacillosis by administration of egg yolk powder from hens immunized with K99-pilated enterotoxigenic Escherichia coli. Am Vet Res 53: 2005-2008.
29. Yokayama H, Peralta RC, Diaz R, Sendo S, Ikemori Y, et al. (1992) Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic Escherichia coli infection in neonatal piglets. Infect Immun 60: 998-1007.
30. Lee SB, Mine Y, Stevenson RM (2000) Effects of hen egg yolk immunoglobulin in passive protection of rainbow trout against Yersinia ruckeri. J Agric Food Chem 48: 110-115.
31. Stevenson RMW, Flett D, Raymond BT (1997) Enteric redmouth (ERM) and other enterobacterial infections of fish. In: Inglis R, Roberts J, Bromage NR (eds.) Bacterial Diseases of Fish V. Blackwell Scientific Publications, Oxford, UK.
32. de Paula VS, da Silva Ados S, de Vasconcelos GA, Iff ET, Silva ME, et al. (2011) Applied biotechnology for production of immunoglobulin Y specific to hepatitis A virus. J Virol Methods 171: 102-106.
33. Lee SH, Lillehoj HS, Park DW, Jang SI, Morales A, et al. (2009) Induction of passive immunity in broiler chickens against Eimeria acervulina by hyperimmune egg yolk immunoglobulin Y. Poult Sci 88: 562-566.