



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Research review paper

## Application of chicken egg yolk immunoglobulins in the control of terrestrial and aquatic animal diseases: A review

Yongping Xu <sup>a,b,c</sup>, Xiaoyu Li <sup>a,b</sup>, Liji Jin <sup>a,b,\*</sup>, Yuhong Zhen <sup>a,d</sup>, Yanan Lu <sup>a</sup>, Shuying Li <sup>e</sup>, Jiansong You <sup>a</sup>, Linhui Wang <sup>a</sup>

<sup>a</sup> School of Life Science and Technology, Dalian University of Technology, Dalian 116024, China

<sup>b</sup> Ministry of Education Center for Food Safety of Animal Origin, Dalian 116620, China

<sup>c</sup> State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China

<sup>d</sup> Department of Pharmacy, Dalian Medical University, Dalian 116044, China

<sup>e</sup> Dalian SEM Bio-Engineering Technology Co. Ltd., Dalian 116620, China

### ARTICLE INFO

#### Article history:

Received 5 August 2010

Revised 29 June 2011

Accepted 11 July 2011

Available online 20 July 2011

#### Keywords:

Egg yolk immunoglobulin

IgY

Terrestrial

Aquatic

Disease control

### ABSTRACT

Oral administration of chicken egg yolk immunoglobulin (IgY) has attracted considerable attention as a means of controlling infectious diseases of bacterial and viral origin. Oral administration of IgY possesses many advantages compared with mammalian IgG including cost-effectiveness, convenience and high yield. This review presents an overview of the potential to use IgY immunotherapy for the prevention and treatment of terrestrial and aquatic animal diseases and speculates on the future of IgY technology. Included are a review of the potential application of IgY for the treatment of livestock diseases such as mastitis and diarrhea, poultry diseases such as *Salmonella*, *Campylobacteriosis*, infectious bursal disease and Newcastle disease, as well as aquatic diseases like shrimp white spot syndrome virus, *Yersinia ruckeri* and *Edwardsiella tarda*. Some potential obstacles to the adoption of IgY technology are also discussed.

© 2011 Elsevier Inc. All rights reserved.

### Contents

1.	Introduction . . . . .	861
2.	Characteristics of chicken immunoglobulin Y (IgY) . . . . .	861
2.1.	Advantages of IgY . . . . .	861
2.2.	Structure and function of chicken IgY . . . . .	861
2.3.	Storage stability of IgY . . . . .	862
3.	Mode of action . . . . .	862
4.	Applications for IgY administration . . . . .	862
4.1.	Application of IgY in the control of livestock diseases . . . . .	862
4.1.1.	Bovine mastitis . . . . .	862
4.1.2.	Diarrhea in piglets . . . . .	863
4.1.3.	Diarrhea in calves . . . . .	864
4.2.	Applications of IgY in the control of poultry diseases . . . . .	864
4.2.1.	Salmonellosis . . . . .	864
4.2.2.	<i>Campylobacteriosis</i> . . . . .	864
4.2.3.	Infectious bursal disease . . . . .	864
4.2.4.	Newcastle disease . . . . .	865
4.3.	Applications of IgY in the control of aquatic diseases . . . . .	865
4.3.1.	Shrimp white spot syndrome virus . . . . .	865
4.3.2.	<i>Y. ruckeri</i> . . . . .	865
4.3.3.	<i>E. tarda</i> . . . . .	865
5.	Limitations of IgY technology . . . . .	866

\* Corresponding author at: School of Life Science and Technology, Dalian University of Technology, Dalian 116024, China. Tel.: +86 411 8470 6359; fax: +86 411 8470 6359.  
E-mail address: [jinlij@dlut.edu.cn](mailto:jinlij@dlut.edu.cn) (L. Jin).

6. Conclusions . . . . .	866
Acknowledgments . . . . .	866
References . . . . .	866

**1. Introduction**

Antibiotics have been used in animal agriculture for growth promotion (sub-therapeutic doses), disease prevention (prophylactic doses) and for the treatment of infection for over 50 years (Turner et al., 2001). Years of research and practical experience have shown that antibiotic use significantly improves animal performance and health status (Cromwell, 2002). However, the use and misuse of in-feed antibiotics have led to problems with drug residues in animal products and increased bacterial resistance (Khachatourians, 1998). As a result, the sub-therapeutic use of antibiotics has been totally banned in European countries since January 2006 (Casewell et al., 2003) and other countries are seriously considering a similar ban. Therefore, alternatives to antibiotics are urgently needed.

A wide range of products have been tested as potential alternatives to antibiotics including organic and inorganic acids (Kim et al., 2005), oligosaccharides (Flickinger et al., 2003), probiotics (Kritas and Morrison, 2005; Taras et al., 2007), herbal extracts (Windisch et al., 2008) and antibodies (Cook, 2004). Among these, oral immunotherapy (passive immunization) with antibodies is a highly attractive and effective alternative approach due to its high specificity. Oral administration with antibodies derived from mammalian serum and colostrum and even monoclonal antibodies have been used successfully (Kuhlman et al., 1988). However, with this technique, it is prohibitively expensive to obtain the large amount of antibody required to prevent and treat disease.

Recently, chicken egg yolk immunoglobulin, referred to as immunoglobulin Y (IgY) has attracted considerable attention as a means to prevent and control disease as it possesses a large number of advantages compared with treatment with mammalian IgG including cost-effectiveness, convenience and high yield (Carlander et al., 2000). Under natural conditions, the serum IgY of laying hens is deposited in large quantities in the egg yolk in order to protect the developing embryo from potential pathogens (Janson et al., 1995). Thus, it is possible to immunize the hen against specific foreign pathogens thereby allowing the production of IgY with activity against these specific disease conditions.

Oral administration of specific IgY antibody has been shown to be effective against a variety of intestinal pathogens such as bovine and human rotaviruses, enterotoxigenic *Escherichia coli* (ETEC), bovine coronavirus, *Salmonella* spp., *Edwardsiella tarda*, *Yersinia ruckeri*, *Staphylococcus* and *Pseudomonas* (Mine and Kovacs-Nolan, 2002). This review presents an overview of the potential to use immunotherapy with specific IgY for the prevention and treatment of terrestrial and aquatic animal diseases and speculates on the future of IgY technology.

**2. Characteristics of chicken immunoglobulin Y (IgY)**

*2.1. Advantages of IgY*

The use of chickens for the production of polyclonal antibodies provides many advantages over production methods using mammals (Table 1). The most significant advantage is that the collection of antibodies is non-invasive. In contrast to conventional methods where animals are often sacrificed in order to collect a sufficient amount of blood to obtain antibodies, production of antibodies in laying hens requires only the collection of eggs, and the high and long-lasting titers produced in chickens reduce the need for frequent

booster injections. Another advantage is that due to the phylogenetic distance between chickens and mammals, chickens often more successfully produce antibodies against highly conserved mammalian proteins than do other mammals and require much less antigen to induce an efficient immune response.

A hen can be considered as a small “factory” for antibody production. A hen usually lays about 300 eggs per year and the egg yolk (15 ml) contains 50–100 mg of IgY of which 2 to 10% are specific antibodies (Rose et al., 1974). Therefore, one immunized hen produces more than 22,500 mg of IgY per year which is equivalent to the production of 4.3 rabbits over the course of a year (Schade et al., 2005). The maintenance costs for keeping hens are also lower than those for mammals such as rabbits (Schade et al., 2005). Therefore, egg yolk provides a more hygienic, cost-efficient, convenient and rich source of antibodies compared with the traditional method of obtaining antibodies from mammalian serum. In contrast to antibiotics, the use of IgY is environmentally-friendly and elicits no undesirable side effects, disease resistance or toxic residues (Coleman, 1999).

*2.2. Structure and function of chicken IgY*

Initially, IgY antibodies were thought to be similar to IgG immunoglobulins, whereas they are now considered to be an evolutionary ancestor to mammalian IgG and IgE and also to IgA (Warr et al., 1995). Although chicken IgY is the functional equivalent of mammalian IgG, there are some profound differences in their structure. The general structure of the IgY molecule is the same as the IgG molecule with two heavy (H) chains and two light (L) chains but IgY has a molecular mass of 180 kDa which is larger than that of mammalian IgG (150 kDa) (Fig. 1). The molecular mass (67–70 kDa) of the H chain in IgY is larger than the H chain from mammals (50 kDa). The greater molecular mass of IgY is due to an increased number of heavy-chain constant domains and carbohydrate chains (Warr et al., 1995). IgG has 3 C regions (C<sub>γ</sub>1–C<sub>γ</sub>3), while IgY has 4 C regions (C<sub>ν</sub>1–C<sub>ν</sub>4) and the presence of one additional C region with its two corresponding carbohydrate chains logically results in a greater molecular mass of IgY compared with IgG.

Other differences in structure include the fact that the hinged region of IgY is much less flexible compared with mammalian IgG. It has also been suggested that IgY is a more hydrophobic molecule than IgG (Davalos-Pantoja et al., 2000). Finally, IgY has an isoelectric point of pH 5.7–7.6, whereas that of IgG lies between 6.1 and 8.5 (Davalos-Pantoja et al., 2000; Sun et al., 2001).

**Table 1**  
Comparison of the characteristics of mammalian IgG and chicken IgY.  
Adapted from Schade et al. (2005).

Parameter	Mammalian IgG	Chicken IgY
Antibody sampling	Invasive	Non-invasive
Source of antibody	Blood serum	Egg yolk
Antibody amount	200 mg IgG/bleed (40 ml blood)	50–100 mg IgY/egg (300 eggs/year)
Frequency of collection	Every two weeks	Every day
Amount of antibody/year	5200 mg	22,500 mg
Amount of specific antibody	~5%	2–10%
Protein–A/G binding	Yes	No
Interference with mammalian IgG	Yes	No
Interference with rheumatoid factor	Yes	No
Activation of mammalian complement	Yes	No

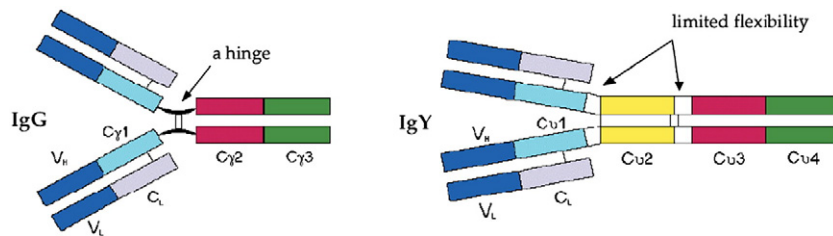


Fig. 1. Structure of IgG and IgY.  
From Narat, 2003.

Unlike mammalian IgG, IgY does not fix mammalian complement and does not interact with mammalian Fc and complement receptors (Carlander et al., 2000). As well, IgY does not bind to protein A, protein G or rheumatoid factor, so no false positives are obtained on immunoassay which is a problem with IgG-based mammalian assays (Davalos-Pantoja et al., 2000). These differences provide significant advantages to the application of IgY technology in many areas of research such as diagnostics (Erhard et al., 2000), antibiotic-alternative therapy (Carlander et al., 2000) and xenotransplantation (Fryer et al., 1999).

### 2.3. Storage stability of IgY

The stability of IgY during storage is reasonably good under specified conditions. IgY solutions may be stored at 4 °C for periods ranging from months to a few years provided 0.02% NaN<sub>3</sub>, 0.03% w/v thimerosal or 50 µg/ml gentamicin has been added to retard microbial growth (Schade et al., 2005). Dried IgY preparations were stored for five to ten years at 4 °C without significant loss of antibody activity and the preparations also retained activity for six months at room temperature and for one month at 37 °C (Larsson et al., 1993). While lyophilization minimizes bacterial growth and maintains better structural integrity of purified IgY, spray-drying is more economical (Yokoyama et al., 1992). Collectively, these unique biological attributes make IgY an effective natural food antimicrobial system and immunotherapeutic agent.

### 3. Mode of action

The exact mechanisms through which IgY counteracts pathogen activity have not been determined. However, several mechanisms have been proposed to express how specific IgY counteracts pathogen activity and these are outlined below.

- (i) **Agglutination.** Tsubokura et al. (1997) suggested that the inhibition of bacterial growth or colonization observed as a result of treatment with IgY can be the result of bacterial agglutination, causing a reduction in CFU rather than actual direct effects on individual bacteria. Although agglutination may be one mediator of growth inhibition, it is unlikely to be the most important mediator because cross-linking of bacteria is precluded by the steric hindrance of the two Fab arms of IgY (Gallagher and Voss, 1974).
- (ii) **Adherence-blockade.** Several *in vitro* studies have suggested that inhibition of adhesion is the dominant mechanism by which specific IgY counteracts pathogen activity (Jin et al. 1998; Lee et al. 2002). Jin et al. (1998) showed that IgY functions to prevent the adherence of *E. coli* K88 to the intestinal mucus of piglets. Particular components exposed on the Gram-negative bacteria surface such as outer membrane protein, lipopolysaccharide, fimbriae (or pili), and flagella, which are crucial factors for bacterial colonization, may be recognized and bound by related polyclonal antibody such as IgY (Sim et al., 2000). This binding may block or impair the

functions of growth-related bacterial components and lead to bacterial growth inhibition. It is also possible that specific IgY binding to bacteria could alter cellular signaling cascades that could result in decreased toxin production and release.

- (iii) **Opsonization followed by phagocytosis.** Nie et al. (2004) determined that IgY improved the phagocytosis of *S. aureus* by neutrophils. Similarly, Zhen et al. (2008b) showed that phagocytic activity of *E. coli* by either milk macrophages or by polymorphonuclear neutrophil leukocytes increased significantly in the presence of IgY. These results suggest that IgY enhanced phagocytic activity. Structural alterations were observed on the surface of *S. typhimurium* (Lee et al., 2002) and *E. coli* O111 (Zhen et al., 2008b) by binding with specific IgY. These changes could be explained by the variation of the electron cloud and/or electric field on the bacterial surface (Lee et al., 2002), resulting in greater vulnerability of the bacterial cells to phagocytosis.
- (iv) **Toxin neutralization.** *S. aureus* capsular is a major virulence factor involved in the onset of bovine mastitis. Wang et al. (2011) studied the effectiveness of IgY against encapsulated *S. aureus*. Their results showed that IgY could prevent *S. aureus* internalization by mammary epithelial cells suggesting toxin neutralization activity, rather than direct growth inhibition as the means through which IgY acts to control mastitis.

## 4. Applications for IgY administration

The potential applications for using orally administered IgY in the control of enteric and non-enteric infections of either bacterial or viral origin in humans and animals have been studied at length and are summarized in Tables 2 and 3.

### 4.1. Application of IgY in the control of livestock diseases

#### 4.1.1. Bovine mastitis

Bovine mastitis is a costly disease for the dairy industry. In the United States alone, the annual costs of mastitis have been estimated to be \$1.5–2.0 billion (\$US). Numerous pathogens can cause mastitis and these can be classified into contagious pathogens (primary *Staphylococcus aureus* and *Streptococcus agalactiae*) or environmental pathogens (primary *E. coli*; Riffon et al., 2001).

In our research, we have reported that specific IgY produced by hens immunized with *S. aureus* and/or *E. coli* was effective in controlling experimental and clinical mastitis (Zhen et al., 2008a,b; 2009). An *in vitro* study conducted in our laboratory indicated that IgY could inhibit the growth of bacteria by altering the bacterial cell surface thereby reducing the ability of bacteria to adhere to mammary epithelial cells (Zhen et al., 2008b). In a further study, the efficacy of specific IgY to reduce clinical and experimental mastitis caused by *S. aureus* was demonstrated by improving milk quality through a decrease in somatic cell and bacterial counts in milk (Zhen et al., 2009). The cure rates resulting from the use of IgY for clinical and experimental mastitis were dramatically higher than for untreated animals (Table 4). Although conducted with very few animals, these

**Table 2**  
Investigations on the use of specific IgY for the control of enteric diseases.  
From Mine and Kovacs-Nolan (2002).

Pathogens	Effects of IgY	Ref.
Rotavirus	Protecting calves from bovine rotavirus-induced diarrhea	Kuroki et al. (1994)
	Preventing murine rotavirus in mice	Yolken et al. (1988)
	Preventing human rotavirus-induced gastroenteritis in mice	Ebina (1996)
Coronavirus	Prevention and treatment of rotavirus-induced gastroenteritis in murine model	Hatta et al. (1993)
	Prevention of rotavirus infection in vitro, using IgY against recombinant HRV coat protein, VP8*	Kovacs-Nolan et al. (2001)
	Protecting neonatal calves from bovine coronavirus (BCV)-induced diarrhea	Ikemori et al. (1997)
<i>Escherichia coli</i>	Preventing K88+, K99+, 987P+ ETEC infection in neonatal piglets	Yokoyama et al. (1992)
	Protecting neonatal calves from fatal enteric colibacillosis by K99-piliated ETEC	Ikemori et al. (1992)
	Inhibiting adhesion of ETEC K88 to piglet intestinal mucus	Jin et al. (1998)
<i>Salmonella</i>	Prevention of ETEC K88+ infection in neonatal and early weaned piglets	Marquardt et al. (1999)
	Protecting mice challenged with <i>S. enteritidis</i> or <i>S. typhimurium</i> from salmonellosis	Yokoyama et al. (1998)
	Preventing fatal salmonellosis in neonatal calves exposed to <i>S. typhimurium</i> or <i>S. dublin</i>	Yokoyama et al. (1998)
<i>Yersinia</i>	Inhibiting adhesion of <i>S. enteritidis</i> to human intestinal cells	Sugita-Konishi et al. (1996)
	Protection of rainbow trout against <i>Y. ruckeri</i> infection	Lee et al. (2000)
<i>Edwardsiella</i>	Preventing Edwardsiellosis of Japanese eels infected with <i>Edwardsiella tarda</i>	Stevenson et al. (1993)
IBDV	Protecting chicks from infectious bursal disease virus	Gutierrez et al. (1993)
<i>Staphylococcus</i>	Inhibiting the production of <i>S. aureus</i> enterotoxin-A	Sugita-Konishi et al. (1996)
<i>Pseudomonas</i>	Inhibiting the growth of <i>P. aeruginosa</i>	Sugita-Konishi et al. (1996)

studies indicate that specific IgY has considerable potential as a therapeutic treatment for mastitis in dairy cows.

#### 4.1.2. Diarrhea in piglets

Enterotoxigenic *E. coli* (ETEC) is by far the most common cause of enteric colibacillosis encountered in neonatal and post-weaned pigs (Yokoyama et al., 1992). The strains of *E. coli* associated with intestinal colonization which cause severe diarrhea are the K88, K99 and 987P fimbrial adhesins. Among the ETEC, those expressing the K88+ fimbrial antigen are the most prevalent forms causing *E. coli* infection world-wide (Rapacz and Hasler-Rapacz, 1986). It has been estimated that K88+ ETEC are responsible for more than half of the piglet mortality which occurs each year (Waters and Sellwood, 1982), causing significant economic loss for the pig industry.

IgY is recognized as an alternative source of antibodies for the prevention of ETEC coli infection because it has been found to inhibit binding of *E. coli* to the intestinal mucosa (Jin et al., 1998). IgY has

**Table 3**  
Investigations on the use of specific IgY in the control of non-enteric diseases.

Pathogens	Effects of IgY	Ref.
<i>Streptococcus mutans</i>	Protecting rats from <i>S. mutans</i> -induced dental caries	Hamada et al. (1991)
Snake venoms	Neutralizing the toxic and lethal components of the venoms	Meenatchisundaram et al. (2008)
SARS coronavirus	Prevention of SARS-coronavirus infection in vitro	Fu et al. (2006)
Avian influenza virus	Protecting birds from avian influenza Subtype H9N2	Rahimi et al. (2007a)

**Table 4**  
Comparison of IgY and penicillin for the treatment of experimental and clinical mastitis caused by *S. aureus*<sup>a</sup>.  
From Zhen et al. (2009).

Treatment	Experimental mastitis <sup>b</sup>		Clinical mastitis <sup>c</sup>	
	No. of mastitic quarters	No. of cured quarters	No. of mastitic quarters	No. of cured quarters
IgY	6	5 (83.3%)*	6	3 (50.0%)*
Penicillin	6	4 (66.7%)*	6	2 (33.3%)*
No treatment	6	0	6	0

\*  $p < 0.05$  compared with no treatment in the same group.

<sup>a</sup> Ten milliliters of IgY, at a concentration of 20 mg ml<sup>-1</sup>, or penicillin, at a concentration of 100 mg ml<sup>-1</sup>, were infused into the mammary glands immediately after the cow's morning and evening milking for six consecutive days.

<sup>b</sup> Mammary glands selected for clinical mastitis treatment met the following criteria: obvious clinical signs of mastitis were detected, *S. aureus* counts of the milk samples were 100–200 CFU ml<sup>-1</sup>, somatic cell counts were more than 5 × 10<sup>5</sup> cells ml<sup>-1</sup>.

<sup>c</sup> Mammary glands selected for inducing experimental mastitis met the following criteria: no clinical signs of mastitis were detected, *S. aureus* counts of the milk were less than 1 × 10<sup>5</sup> CFU ml<sup>-1</sup> and no other bacteria were detected, somatic cell counts were less than 1 × 10<sup>5</sup> CFU ml<sup>-1</sup>. Experimental mastitis was induced by mammary infusion with 10<sup>7</sup> CFU ml<sup>-1</sup> of *S. aureus*.

been orally administered to piglets and offers a potential prophylactic and therapeutic approach for controlling *E. coli*-induced diarrhea. Yokoyama et al. (1992) showed that orally administered IgY generated against *E. coli* K88, K99, or 987P fimbriae was protective against infection from each of the three homologous strains of *E. coli* in a dose-dependent manner. *E. coli* K88, K99, and 987P strains adhered equally to porcine epithelial cells from the duodenum and ileum but failed to do so in the presence of homologous anti-fimbrial IgY.

A group of researchers at the University of Manitoba (Winnipeg, Canada) have carried out some excellent studies on the passive protective effect of IgY against ETEC K88 fimbriae in the control of neonatal and early-weaned piglets in vitro and in vivo (Jin et al., 1998; Marquardt et al., 1999). In an animal feeding study, 21-day old pigs were challenged with 5 ml of ETEC K88 at a dose of 10<sup>12</sup> CFU ml<sup>-1</sup> per piglet. IgY was administered to piglets three times a day for two days. Control piglets (treated with IgY from non-immunized hens) developed severe diarrhea within 12 h and 30% of the pigs died. In contrast, the pigs given IgY from immunized hens exhibited no signs of diarrhea when observed 24 and 48 h after treatment (Table 5). Furthermore, the field study showed the suppression of the incidence and severity of diarrhea in 14–18-day-old weaned piglets fed specific IgY powder, which was much lower than in those fed a commercial diet containing an antibiotic. The number of pigs in this study was not large and it would be desirable to repeat this study with a greater number of animals.

**Table 5**  
Clinical response of 21-day-old pigs after challenge with ETEC K88+ MB and treatment with egg-yolk antibodies.  
From Marquardt et al. (1999).

Treatment <sup>a</sup>	No. of pigs	No. of pigs with diarrhea (FC score <sup>b</sup> ) after			Weight gain (g)	% of pigs dead
		24 h	48 h	72 h		
<i>E. coli</i> challenged	10	3/10 (1.0)	8/10 (2.0)	4/7 (3.0)	-36.2	30.0%
<i>E. coli</i> challenged + IgY	10	5/10 (1.6)	0/10 (0.0)	1/10 (0.5)	+90.6	0.0%

<sup>a</sup> All the 21-day-old piglets were challenged orally two times (at 0 and 5 h) with a dose of 10<sup>12</sup> CFU ml<sup>-1</sup> of viable organism per piglet. Piglets in the *E. coli* challenged + IgY group were treated with 0.5 g of egg yolk antibodies, three times a day (-1, 4 and 9 h after the first ETEC challenge) for the first day and once a day for the next two days after the first ETEC challenge.

<sup>b</sup> FC score is the mean fecal consistency score: 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. Pigs with a fecal score of ≤1 were considered not to have diarrhea.

EPEC can cause severe diarrhea in young animals by proliferation in the small intestine and through the production of two types of enterotoxins including a heat-labile enterotoxin (LT) and a family of heat-stable enterotoxins (ST) that includes STa and STb (Dubreuil, 1997). These enterotoxins are crucial virulence factors and therefore effective programs for immunological protection against EPEC-mediated diarrhea should include antitoxic immunity (Cardenas and Clements, 1993). Because of their small size, enterotoxins STa and STb are poorly immunogenic, but they can attain immunogenicity when coupled chemically or genetically to an appropriate carrier (Clements, 1990; Dubreuil et al., 1996).

Antitoxic antibodies were shown to be important in the prevention of *E. coli*-induced diarrhea. Considering the limited number of classes of enterotoxins, enterotoxin-based vaccines should provide broad-spectrum protection against various EPEC. In order to make the useful toxoids, polyvalent enterotoxin STa–LTB–STb DNA and protein vaccines endowed immunogenicity to both STa and STb were constructed (You et al., 2011). We immunized laying hens with DNA vaccines and obtained antitoxic antibodies from egg yolks and this was confirmed by indirect ELISA. The polyvalent DNA vaccine pCI–STa–LTB–STb expressed the STa–LTB–STb fusion peptide in vitro in cultured HeLa cells. These egg yolk antibodies were able to neutralize the biological activity of ST and LT (You, 2011). Therefore, the trivalent fusion enterotoxin STa–LTB–STb has the potential to serve as a useful toxin-based vaccine which can provide broad protection against EPEC-induced diarrhea.

#### 4.1.3. Diarrhea in calves

Neonatal calf diarrhea caused by bovine rotavirus (BRV) is a common disease and causes high mortality in cattle (Lee et al., 1995). Anti-BRV IgY has been shown to successfully provide protection against BRV infection in calves (Kuroki et al., 1994).

Bovine coronavirus (BCV) is an important enteric pathogen that is responsible for both neonatal calf diarrhea and acute diarrhea in adult cattle. BCV may be more severe than BRV as it replicates in the epithelium of both the small and large intestine whereas BRV infects only the small intestine. Ikemori et al. (1997) examined the efficacy of specific IgY antibodies and cow colostrum antibodies to BCV-induced diarrhea in calves. They found that control calves which received no antibodies developed severe diarrhea resulting in all calves dying within 6 days of BCV challenge whereas calves treated with milk containing egg yolk or colostrum had positive weight gains and none of these calves died (Table 6). This study was conducted with very few animals and a larger study should be conducted to confirm these findings. However, these results do indicate that orally administered egg yolk or colostrum antibodies have the potential to passively protect calves against BCV infection and the effect of IgY was higher than that of colostrum alone indicating that the passive immunization strategy with IgY provided a more efficacious alternative to existing treatments for BCV. The passive protective effects of using anti-EPEC IgY administration on fatal enteric colibacillosis in neonatal calves have also been studied (Ikemori et al., 1992). Calves fed milk containing IgY had only transient diarrhea, 100% survival and exhibited good body weight gains during the course of the study. In contrast, the controls

**Table 6**

Clinical response of calves to challenge exposure with bovine coronavirus and subsequent treatment with egg yolk powder. From Ikemori et al. (1997).

Group	Antibody titer	No. diarrhea/ total no. (%)	No. dead/ total no. (%)	Cumulative fecal score	Weight gain (%)
Control	–	4/4 (100)	4/4 (100)	30.5 ± 6.1	–7.4 ± 2.3
Egg powder	1280	3/4 (75)	2/4 (50)	20.0 ± 7.4	–5.1 ± 10.4
Egg powder	2560	3/4 (50)	0/4 (0)*	3.8 ± 3.8**	6.3 ± 2.4**

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

**Table 7**

Effect of specific IgY on the concentration of *Salmonella* in the cecum of broiler chickens. From Rahimi et al. (2007b).

Age of bird	Log <sub>10</sub> <i>Salmonella</i> per gram cecal contents	
	Control	IgY
Day 21	5.26 <sup>b</sup>	1.23 <sup>a</sup>
Day 28	3.98 <sup>b</sup>	0.27 <sup>a</sup>

<sup>a,b</sup> Means in the same row lacking a common superscript differ significantly ( $p < 0.01$ ).

which received no antibody had severe diarrhea and all died within 6 days of infection.

## 4.2. Applications of IgY in the control of poultry diseases

### 4.2.1. Salmonellosis

*Salmonella* infections are thought to be responsible for a variety of acute and chronic diseases of poultry. It has been shown that specific IgY against *Salmonella enteritidis* or *Salmonella typhimurium* inhibits bacterial growth in vitro (Lee et al., 2002). Studies have demonstrated that egg derived anti-*S. enteritidis* IgY antibody had a beneficial effect in reducing the colonization of *Salmonella* in market-aged broilers (Rahimi et al., 2007b; Tellez et al., 2001) (Table 7). Whether or not this translates into improvements in broiler performance has not been determined.

The use of whole egg powder (containing antibody) as a feed additive may be an alternative way to reduce the rate of *Salmonella* contamination of eggs. Gurtler et al. (2004) found that in the experimental group, 13.3% of the eggs were *Salmonella* positive, which was significantly lower than the 29.4% *Salmonella*-positive eggs in the control group ( $p \leq 0.001$ ) (Table 8). However, it must be pointed out that the majority of the *Salmonella* was found on the shell and there were only modest differences in *Salmonella* numbers in the albumen and yolk. Whether or not this translates into any less health risk for consumers of egg products is not known at this time.

### 4.2.2. Campylobacteriosis

*Campylobacter jejuni* has become a major concern to the commercial broiler, turkey and commercial egg-producing flocks in all countries. Tsubokura et al. (1997) used egg yolk antibodies for prophylactic and therapeutic applications in *Campylobacter*-infected chickens. In a prophylaxis experiment, it was found that these antibodies caused a 99% decrease in the number of *Campylobacter* observed, whereas in a therapy trial (antibodies were given after establishment of the infection), the number of bacteria in the feces was 80–95% lower. Unfortunately, bird performance was not monitored in this study.

### 4.2.3. Infectious bursal disease

Infectious bursal disease (IBD) is an acute, highly contagious disease of young chickens caused by IBD virus (Chettle et al., 1989). Early sub-clinical infections which usually result in immunosuppression are the most important form of this disease because of the large economic loss that results from their presence. Chickens immunosuppressed by early IBD infections do not respond well to vaccination

**Table 8**

Rate of *Salmonella* isolation in eggs laid by hens after oral application of IgY egg powder. From Gurtler et al. (2004).

Parts	% Eggs with <i>Salmonella</i>		
	IgY	Control	p value
Whole eggs	13.3	29.4	$p \leq 0.001$
Egg shell	13.3	29.2	$p \leq 0.001$
Albumen	0.3	0.5	n.s.
Yolk	0.6	2.4	$p \leq 0.05$

and show increased susceptibility to other diseases such as bacterial infection with *E. coli* or viral infections like bronchitis or inclusion body hepatitis (Muhammad et al., 1996). Antibiotic therapy is the most readily-available approach for controlling IBD-induced secondary bacterial infections in affected flocks. It has been shown that specific IgY has a great potential as an alternative to antibiotics for IBD. Muhammad et al. (2001) demonstrated that yolks from hyper-immunized hens can be used to control IBD in commercial laying hens. The IBD infected broilers (28 days old) treated with the yolk induced 80% recovery while all the control (untreated) birds died.

#### 4.2.4. Newcastle disease

Newcastle disease is a severe viral infection causing a respiratory nervous disorder in several species of poultry including chickens and turkeys. This disease is endemic in commercial poultry from many countries and can cause great economic loss due to high mortality rates (Lancaster, 1976). Vaccination has been used to prevent this disease in endemic areas, but vaccines are not always effective and vaccinated flocks may still be infected. When outbreaks of Newcastle disease occur in a commercial poultry flock, antibiotics may be given to prevent secondary bacterial infections which are usually triggered by this disease. It has been shown that egg yolk antibodies conferred protection in chickens against Newcastle disease (Box et al., 1969; Wills and Luginbuhl, 1963). Wills and Luginbuhl (1963) have found that subcutaneous administration of egg yolk containing high levels of IgY antibody specific to Newcastle disease protected 80% of the birds during a four-week study period.

### 4.3. Applications of IgY in the control of aquatic diseases

#### 4.3.1. Shrimp white spot syndrome virus

White spot syndrome virus (WSSV) is a virulent pathogen causing high mortality and significant economic loss in cultured shrimp operations worldwide (Wongteerasupaya et al., 1995). As with other invertebrates, shrimp lack a true adaptive immune response system (Kimbrell and Beutler, 2001). However, shrimp may exhibit a quasi-immune response which possibly shows WSSV-specific effects (Witteveldt et al., 2004; Wu et al., 2002). WSSV can be neutralized by chicken IgY produced against a truncated fusion protein of VP28 and VP19 (Kim et al., 2004) and therefore, passive immunization with IgY against WSSV has potential for immunotherapeutic application to prevent WSSV infection in shrimp.

Some researchers have demonstrated passive immunization by anti-viral egg yolk antibodies (IgY) (Arasteh et al., 2004; Kweon et al., 2000). However, our research work (Lu et al., 2008) represents the first report of the passive immunization of shrimp with IgY obtained from the egg yolk of chickens vaccinated with a DNA vaccine comprising genes encoding structural proteins of WSSV or with inactivated WSSV. Our work showed that IgY antibodies have a high affinity for WSSV and maintained good biological and neutralization activity especially when induced by the traditional inactivated vaccine. IgY antibody induced by the DNA vaccine was also able to neutralize disease in shrimp experimentally challenged with WSSV but was still inferior to inactivated WSSV (Lu et al., 2008). Using specific IgY from hens immunized with inactivated WSSV and DNA vaccine to neutralize WSSV, the challenged shrimp had 73.3 and 33.3% survival, respectively (Fig. 2). As well, the effectiveness of IgY provided by intramuscular injection, oral administration, and immersion was investigated in crayfish (*Procambarus clarkii*) challenged with WSSV (Lu et al., 2009). Our results showed that immunization of specific IgY antibodies through intramuscular injection, oral administration or immersion were all effective means of protecting crayfish against WSSV. Unfortunately, these are labor intensive techniques and may have limited application under commercial aquaculture conditions where crayfish are raised in the ocean.

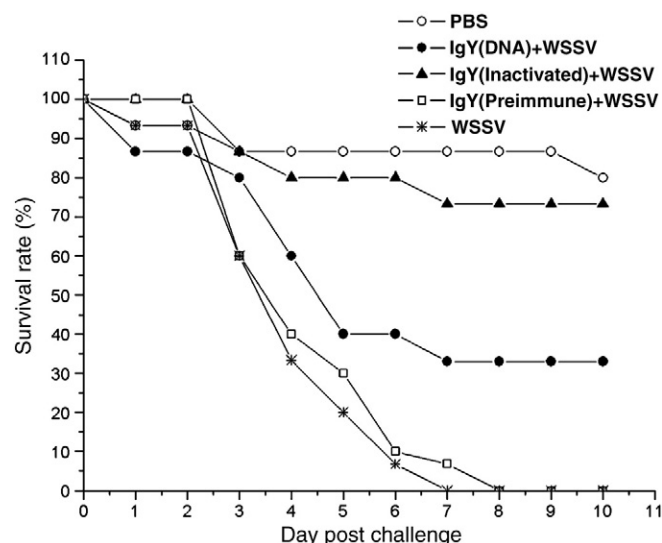


Fig. 2. Effect of IgY on the survival rate of shrimp challenged with white spot syndrome virus.

From Lu et al., 2008.

Egg yolk antibodies can be harvested in a more cost efficient and convenient manner compared with production of antibody from mammals (Mine and Kovacs-Nolan, 2002). IgY in egg yolk could be prepared in different forms such as egg yolk powder, purified powder and liquor which may provide a feasible and significant way forward in research using antibodies against WSSV.

#### 4.3.2. *Y. ruckeri*

The effectiveness of IgY in passive immunization of rainbow trout against *Y. ruckeri* infection has been studied (Lee et al., 2000). *Y. ruckeri* is the aetiological agent of enteric red mouth disease, a systemic bacterial septicaemia, principally affecting farmed rainbow trout, *Oncorhynchus mykiss* (Stevenson et al., 1993). *Y. ruckeri* could persist for long periods in carrier fish and are shed in feces, representing a continuing source of infection. If a population of asymptomatic carrier fish could be adequately cleared by oral administration of anti-*Y. ruckeri* antibody, such treatment might be a cost-effective alternative approach to the slaughter of a stock of fish with a potential health risk. Rainbow trout fed with anti-*Y. ruckeri* IgY, 2 h before an immersion challenge with *Y. ruckeri*, showed a lower mortality rate after 8 days than did fish fed unsupplemented IgY feed before the challenge (Lee et al., 2000). The fish fed IgY also appeared to have fewer infections after 8 days, based on organ and intestinal cultures. In a subsequent trial, the IgY-fed fish had lower mortality than fish receiving unsupplemented IgY feed (Lee et al., 2000). The percentage of IgY-fed fish carrying *Y. ruckeri* in their intestinal samples appeared to be lower than in the untreated controls, regardless of whether the IgY containing feed was given before or after the challenge. The oral administration of specific IgY against fish pathogens could provide an alternative method to antibiotics and chemotherapy for prevention of bacterial diseases of fish in fish farm.

#### 4.3.3. *E. tarda*

*E. tarda* is another important fish pathogen which is spread by infection through the intestinal mucosa (Noga, 1996). Edwardsiellosis in Japanese eels has become a serious problem for the eel farming industry (Wakabayashi and Egusa, 1973). Egg yolk antibodies have been investigated for the prevention of this infectious disease because the use and misuse of antibiotics has led to growing problems with drug residues in fish products and increased bacterial resistance (Gutierrez et al., 1993; Hatta et al., 1994). Anti-*E. tarda* IgY were simultaneously administered orally to Japanese eels (*Anguilla*

*japonica*) with the pathogenic bacteria ( $10^5$ – $10^6$  CFU per fish). The non-treated infected eels died within 15 days whereas the eels receiving IgY survived and no disease symptoms were observed, suggesting that anti-*E. tarda* IgY is effective in preventing edwardsiellosis (Gutierrez et al., 1993). Again limited numbers of eels were used in this study and a repeat with greater numbers would certainly be welcomed.

## 5. Limitations of IgY technology

There are limitations to the use of IgY for oral passive immunotherapy due to its susceptibility to proteolysis. IgY is fairly resistant to digestion by intestinal proteases (Hatta et al., 1993; Shimizu et al., 1988). However, it was found that the activity of IgY was decreased at pH 3.5 and completely lost at pH 3.0 (Shimizu et al., 1988). IgY has a serum half-life of 1.85 days in newborn pigs. This is shorter than the reported serum half-life of 12 to 14 days for homologous IgG (colostral antibodies). The fraction of IgY absorbed into the circulation when administered in pigs decreased with increasing age of pigs (Yokoyama et al., 1993).

Since the primary target site of IgY is the small intestine, in order for it to function it is essential to find an effective method to protect IgY against peptic digestion and acidity in the stomach. It has been shown that microencapsulation can be an effective method for protecting IgY from gastric inactivation (Chang et al., 2002; Cho et al., 2005; Kovacs-Nolan and Mine, 2005; Li et al., 2007, 2009), but this process will undoubtedly add additional costs. Additional study will be needed before microencapsulation can be applied under commercial conditions.

In vitro studies have demonstrated that IgY is effective against many Gram-negative bacteria. In contrast, inhibition activity of IgY on Gram-positive bacteria has only occasionally been reported (Sugita-Konishi et al., 1996; Zhen et al., 2008a, 2009; Wang et al., 2011). This may be due to the fact that Gram-positive bacteria have a more compact cell wall compared with Gram-negative bacteria. However, this clearly limits the types of diseases that can be treated with IgY technology.

The objective to treatment with IgY is not always prevention of a disease but sometimes simply a reduction of infectious agents (Kuroki et al., 1993). Unlike mammalian IgG, IgY does not bind complement or acts as an opsonin in phagocytosis. Therefore, IgY are unable to kill bacteria directly. It was also shown that IgY antibodies were not able to displace *E. coli* K88+ MB once bound to the mucosal receptor. This indicates that the antibodies have a lower affinity for the receptor than that of K88+ fimbriae. Since IgY could not remove previously bound *E. coli* K88+ MB from the mucus, a prophylactic use for IgY is suggested (Jin et al., 1998). However, therapeutic effects of IgY in the control of infections have been reported, such as *E. coli* K88 in piglets (Yokoyama et al., 1992; Wiedemann et al., 1991), *C. jejuni* in chickens (Tsubokura et al., 1997) and *Salmonella* in mice (Yokoyama et al., 1998).

At present, the production cost of high quality IgY antibodies is higher than the cost of routine antibiotics (Casadevall and Scharff, 1994). Therefore, the development of methods for large-scale production of IgY with high recovery and purity are needed. The method should be simple, economical and requires few chemicals. In addition, there is no consensus on the most suitable IgY extraction method for commercial application (Schade et al., 2005) and this requires further study.

## 6. Conclusions

Oral administration of chicken egg yolk immunoglobulin appears to have considerable potential as a means of controlling infectious diseases of bacterial and viral origin and may be the disease treatment of the future. To date, IgY technology has been used for the treatment of livestock diseases such as mastitis and diarrhea, poultry diseases

such as *Salmonella*, *Campylobacteriosis*, infectious bursal disease and Newcastle disease, as well as aquatic diseases like shrimp white spot syndrome virus, *Y. ruckeri* and *E. tarda*. Some advantages of IgY in the control of animal diseases are:

1. They are highly effective.
2. They are cost effective.
3. Collection of eggs is non-invasive.
4. The treatment is safe and live organisms are not used.
5. The procedure is environmentally friendly.
6. No toxic residues are produced and there is no development of resistance.
7. The treatment can be used to control many different types of pathogens.

## Acknowledgments

The work from our laboratory that is reviewed here was supported by funds from National Natural Science Foundation of China (30371053; 30871806; 31001053). The authors gratefully thank Professor Philip A. Thacker from University of Saskatchewan for his valuable suggestion and for revising the manuscript.

## References

- Arasteh N, Aminirissehei AH, Yousif AN, Albright LJ, Durance TD. Passive immunization of rainbow trout (*Oncorhynchus mykiss*) with chicken egg yolk immunoglobulins (IgY). *Aquaculture* 2004;231:23–36.
- Box PG, Stedman RA, Singleton L. Newcastle Disease: I. The use of egg yolk derived antibody for passive immunisation of chickens. *J Comp Pathol* 1969;79:495–506.
- Carlander D, Kollberg H, Wejaker PE, Larsson A. Peroral immunotherapy with yolk antibodies for the prevention and treatment of enteric infections. *Immunol Res* 2000;21:1–6.
- Cardenas L, Clements JD. Development of mucosal protection against the heat-stable enterotoxin (ST) of *Escherichia coli* by oral immunization with a genetic fusion delivered by a bacterial vector. *Infect Immunol* 1993;61:4629–36.
- Casadevall A, Scharff MD. Serum therapy revisited: Animal models of infection and development of passive antibody therapy. *Antimicrob Agents Chemother* 1994;38:1695–702.
- Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother* 2003;52:159–61.
- Chang HM, Lee YC, Chen CC, Tu YY. Microencapsulation protects immunoglobulin in yolk (IgY) specific against *Helicobacter pylori* urease. *J Food Sci* 2002;67:15–20.
- Chettle NJ, Stuart C, Wyeth PJ. Outbreak of virulent infectious bursal disease in East Anglia. *Vet Rec* 1989;125:271–2.
- Cho YH, Lee JJ, Park IB, Huh CS, Baek YJ, Park J. Protective effect of microencapsulation consisting of multiple emulsification and heat gelation processes on immunoglobulin in yolk. *J Food Sci* 2005;70:148–51.
- Clements JD. Construction of a nontoxic fusion peptide for immunization against *Escherichia coli* strains that produce heat-labile and heat-stable enterotoxins. *Infect Immun* 1990;58:1159–66.
- Coleman M. Using egg antibodies to treat diseases. In: Sim JS, Nakai S, Guenter W, editors. *Egg Nutr Biotechnol*. Wallingford: CAB International; 1999. p. 351–70.
- Cook ME. Antibodies: alternatives to antibiotics in improving growth and feed efficiency. *Appl Poult Res* 2004;13:106–19.
- Cromwell GL. Why and how antibiotics are used in swine production. *Anim Biotechnol* 2002;13:7–27.
- Davalos-Pantoja L, Ortega-Vinuesa JL, Bastos-Gonzalez D, Hidalgo-Alvarez R. A comparative study between the adsorption of IgY and IgG on latex particles. *Biomater Sci Polym Ed* 2000;11:657–73.
- Dubreuil JD, Letellier A, Harel J. A recombinant *Escherichia coli* heat-stable enterotoxin b (STb) fusion protein eliciting neutralizing antibodies. *FEMS Immunol Med Mic* 1996;13:317–23.
- Dubreuil JD. *Escherichia coli* STb enterotoxin. *Micro* 1997;143:1783–95.
- Ebina T. Prophylaxis of rotavirus gastroenteritis using immunoglobulin. *Arch Virol Suppl* 1996;12:217–23.
- Erhard MH, Schmidt P, Zinsmeister P, Hofmann A, Munster U, Kaspers B. Adjuvant effects of various lipopeptides and interferon-gamma on the humoral immune response of chickens. *Poult Sci* 2000;79:1264–70.
- Flickinger EA, Van Loo J, Fahey GC. Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals: a review. *Crit Rev Food Sci Nutr* 2003;43:19–60.
- Fryer J, Firca J, Leventhal J, Blondin B, Malcolm A, Ivancic D, et al. IgY antiporcine endothelial cell antibodies effectively block human antiporcine xenoantibody binding. *Xenotransplantation* 1999;6:98–109.
- Fu CY, Huang H, Wang XM, Liu YG, Wang ZG, Cui SJ, et al. Preparation and evaluation of anti-SARS coronavirus IgY from yolks of immunized SPF chickens. *J Virol Methods* 2006;133:112–5.



- Gallagher JS, Voss EW. Conformational state of chicken 7S immunoglobulin. *Immunochem* 1974;11:461–5.
- Gurtler M, Methner U, Kobilke H, Fehlhaber K. Effect of orally administered egg yolk antibodies on *Salmonella enteritidis* contamination of hen's eggs. *J Vet Med B Infect Dis Vet Public Health* 2004;51:129–34.
- Gutierrez MA, Miyazaki T, Hatta H, Kim M. Protective properties of egg yolk IgY containing anti-*Edwardsiella tarda* antibody against paracolo disease in the Japanese eel, *Anguilla japonica* Temminck & Schlegel. *J Fish Dis* 1993;16:113–22.
- Hameda S, Horikoshi T, Minami T, Kawabata S, Hiraoka J, Fujiwara T, et al. Oral passive immunization against dental caries in rats by use of hen egg yolk antibodies specific for cell-associated glucosyltransferase of *Streptococcus mutans*. *Infect Immun* 1991;59:4161–7.
- Hatta H, Tsuda K, Akachi S, Kim M, Yamamoto T, Ebina T. Oral passive immunization effect of anti-human rotavirus IgY and its behavior against proteolytic enzymes. *Biosci Biotechnol Biochem* 1993;57:1077–81.
- Hatta H, Mabe K, Kim M, Yamamoto T, Gutierrez MA, Miyazaki T. Prevention of fish disease using egg yolk antibody. In: Sim JS, Nakai S, editors. *Egg Uses and Processing Technologies: New Developments*. Wallingford: CAB International; 1994. p. 241–9.
- Ikemori Y, Kuroki M, Peralta RC, Yokoyama H, Kodama Y. Protection of neonatal calves against fatal enteric colibacillosis by administration of egg yolk powder from hens immunized with K99-piliated enterotoxigenic *Escherichia coli*. *Am Vet Res* 1992;53:2005–8.
- Ikemori Y, Ohta M, Umeda K, Icatlo FC, Kuroki M, Yokoyama H, et al. Passive protection of neonatal calves against bovine coronavirus-induced diarrhea by administration of egg yolk or colostrum antibody powder. *Vet Microbiol* 1997;58:105–11.
- Janson AK, Smith CI, Hammarstrom L. Biological properties of yolk immunoglobulins. *Adv Exp Med Biol* 1995;371:685–90.
- Jin LZ, Samuel K, Baidoo K, Marquardt RR, Frohlich AA. *In vitro* inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 to piglet intestinal mucus by egg yolk antibodies. *FEMS Immunol Med Microbiol* 1998;21:313–21.
- Khachatourians GG. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Can Med Assoc J* 1998;159:1129–36.
- Kim DK, Jang IK, Seo HC, Shin SO, Yang SY, Kim JW. Shrimp protected from WSSV disease by treatment with egg yolk antibodies (IgY) against a truncated fusion protein derived from WSSV. *Aquaculture* 2004;237:21–30.
- Kim YY, Kil DY, Oh HK, Han IK. Acidifier as an alternative material to antibiotics in animal feed. *Asian-Aust J Anim Sci* 2005;18:1048–60.
- Kimbrell DA, Beutler B. The evolution and genetics of innate immunity. *Nat Rev Genet* 2001;2:256–67.
- Kovacs-Nolan J, Sasaki E, Yoo D, Mine Y. Cloning and expression of human rotavirus spike protein, VP8\* in *Escherichia coli*. *Biochem Biophys Res Commun* 2001;282:1183–8.
- Kovacs-Nolan J, Mine Y. Microencapsulation for the gastric passage and controlled intestinal release of immunoglobulin Y. *J Immunol Methods* 2005;296:199–209.
- Kritas SK, Morrison RB. Evaluation of probiotics as a substitute for antibiotics in a large pig nursery. *Vet Rec* 2005;56:447–8.
- Kuhlman R, Wiedemann V, Schmidt P, Wanke R, Linckh E, Losch U. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. I. Immunization and antibody determination. *J Vet Med B* 1988;35:610–6.
- Kuroki M, Ikemori Y, Yokoyama H, Peralta RC, Icatlo Jr FC, Kodama Y. Passive protection against bovine rotavirus-induced diarrhea in murine model by specific immunoglobulins from chicken egg yolk. *Vet Microbiol* 1993;37:135–46.
- Kuroki M, Ohta Y, Ikemori Y, Peralta RC, Yokoyama H, Kodama Y. Passive protection against bovine rotavirus in calves by specific immunoglobulins from chicken egg yolk. *Arch Virol* 1994;138:143–8.
- Kweon CH, Kwon BJ, Woo SR, Kim JM, Woo GH, Son DH, et al. Immunoprophylactic effect of chicken egg yolk immunoglobulin (IgY) against porcine epidemic diarrhea virus (PEDV) in piglets. *J Vet Med Sci* 2000;62:961–4.
- Lancaster JE. A history of Newcastle disease with comments on its economic effects. *World Poult Sci J* 1976;32:167–75.
- Larsson A, Balow R, Lindahl TL, Forsberg P. Chicken antibodies: taking advantage of evolution—a review. *Poult Sci* 1993;72:1807–12.
- Lee J, Babiuk LA, Harland R, Gibbons E, Elazhary Y, Yoo D. Immunological response to recombinant VP8\* subunit protein of bovine rotavirus in pregnant cattle. *J Gen Virol* 1995;76:2477–83.
- Lee SB, Mine Y, Stevenson RMW. Effects of hen egg yolk immunoglobulin in passive protection of rainbow trout against *Yersinia ruckeri*. *J Agric Food Chem* 2000;48:110–5.
- Lee EN, Sunwoo HH, Menninen K, Sim JS. *In vitro* studies of chicken egg yolk antibody (IgY) against *Salmonella enteritidis* and *Salmonella typhimurium*. *Poult Sci* 2002;81:632–41.
- Li XY, Jin LJ, McAllister TA, Stanford K, Xu JY, Lu YN, et al. Chitosan–alginate microcapsules for oral delivery of egg yolk immunoglobulin (IgY). *J Agric Food Chem* 2007;55:2911–7.
- Li XY, Jin LJ, Uzonna JE, Li SY, Liu JJ, Li HQ, et al. Chitosan–alginate microcapsules for oral delivery of egg yolk immunoglobulin (IgY): *in vivo* evaluation in a pig model of enteric colibacillosis. *Vet Immunol Immunopharmacol* 2009;129:132–6.
- Lu YN, Liu JJ, Jin LJ, Li XY, Zhen YH, Xue HY, et al. Passive protection of shrimp against white spot syndrome virus (WSSV) using specific antibody from egg yolk of chickens immunized with inactivated virus or a WSSV–DNA vaccine. *Fish Shellfish Immunol* 2008;25:604–10.
- Lu YN, Liu JJ, Jin LJ, Li XY, Zhen YH, Xue HY, et al. Passive immunization of crayfish (*Procambarus clarkii*) with chicken egg yolk immunoglobulin (IgY) against white spot syndrome virus (WSSV). *Appl Biochem Biotechnol* 2009;159:750–8.
- Marquardt RR, Jin LZ, Kim JW, Fang L, Frohlich AA, Baidoo SK. Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early-weaned piglets. *FEMS Immunol Med Microbiol* 1999;23:283–8.
- Meenachisundaram S, Parameswari G, Michael A, Ramalingam S. Neutralization of the pharmacological effects of cobra and krait venoms by chicken egg yolk antibodies. *Toxicon* 2008;52:221–7.
- Mine Y, Kovacs-Nolan J. Chicken egg yolk antibodies as therapeutics in enteric infectious disease: a review. *J Med Food* 2002;5:159–69.
- Muhammad K, Anwar MS, Yaqub MT. Failure of vaccines to control infectious bursal disease in commercial poultry. *Pak Vet J* 1996;16:119–21.
- Muhammad K, Rabbi F, Hussain I, Riaz A, Saeed K, Hussain T. Passive immunization against infectious bursal disease in chicks. *Int J Agric Biol* 2001;3:413–5.
- Narat M. Production of antibodies in chickens. *Food Technol Biotechnol* 2003;41:259–67.
- Nie R, Wu D, Hu G, Zhang J, Yang H, Wen Z. Effect of specific egg yolk immunoglobulins on phagocytosis by neutrophils. *Chin J Vet Med* 2004;12:23–5.
- Noga EJ. *Fish disease: diagnosis and treatment*. St. Louis: Mosby-Year Book Inc.; 1996. p. 147–8.
- Rahimi S, Salehifar E, Ghorashi SA, Grimes JL, Torshizi MAK. The effect of egg-derived antibody on prevention of avian influenza subtype H9N2 in layer chickens. *Int J Poult Sci* 2007a;6:207–10.
- Rahimi S, Salehifar E, Ghorashi SA, Grimes JL, Torshizi MAK. Prevention of *Salmonella* infection in poultry by specific egg-derived antibody. *Int J Poult Sci* 2007b;6:230–5.
- Rapacz J, Hasler-Rapacz J. Polymorphism and inheritance of swine small intestinal receptors mediating adhesion of three serological variants of *Escherichia coli*-producing K88 pilus antigen. *Anim Genet* 1986;17:305–21.
- Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M, Lagace J. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. *J Clin Microbiol* 2001;39:2584–9.
- Rose ME, Orlans E, Buttress N. Immunoglobulin classes in the hen's eggs: their segregation in yolk and white. *Eur J Immunol* 1974;4:521–3.
- Schade R, Calzado EG, Sarmiento R, Chacana PA, Porankiewicz-Asplund J, Terzolo HR. Chicken egg yolk antibodies (IgY-technology): a review of progress in production and use in research and human and veterinary medicine. *Altern Lab Anim* 2005;33:129–54.
- Shimizu M, Fitzsimmons RC, Nakai S. Anti-*E. coli* immunoglobulin Y isolated from egg yolk of immunized chickens as a potential food ingredient. *J Food Sci* 1988;53:1360–6.
- Sim JS, Sunwoo HH, Lee EN. Ovoglobulin IgY. In: Naidu AS, editor. *Natural food antimicrobial systems*. New York: CRC Press; 2000. p. 227–52.
- Stevenson RMW, Flett D, Raymond BT. Enteric redmouth (ERM) and other enterobacterial infections of fish. In: Inglis R, Roberts J, Bromage NR, editors. *Bacterial diseases of fish*. Oxford: Blackwell Scientific Publications; 1993. p. 80–105.
- Sugita-Konishi Y, Shibata K, Yun SS, Hara-Kudo Y, Yamaguchi K, Kumagai S. Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria. *Biosci Biotechnol Biochem* 1996;60:886–8.
- Sun S, Mo W, Ji Y, Liu S. Preparation and mass spectrometric study of egg yolk antibody (IgY) against rabies virus. *Rapid Commun Mass Spectrom* 2001;15:708–12.
- Taras D, Vahjen W, Simon O. Probiotics in pigs—modulation of their intestinal distribution and of their impact on health and performance. *Livest Sci* 2007;108:229–31.
- Tellez G, Petrone VM, Escorcia M, Morishita TY, Cobb CW, Villaseñor L, et al. Evaluation of avian-specific probiotic and *Salmonella enteritidis*-, *Salmonella typhimurium*-, and *Salmonella heidelberg*-specific antibodies on cecal colonization and organ invasion of *Salmonella enteritidis* in broilers. *J Food Prot* 2001;64:287–91.
- Tsubokura K, Berndtson E, Bogstedt A, Kaijser B, Kim M, Ozeki M, et al. Oral administration of antibodies as prophylaxis and therapy in *Campylobacter jejuni*-infected chickens. *Clin Exp Immunol* 1997;108:451–5.
- Turner JL, Dritz SS, Minton JE. Review: alternatives to conventional antimicrobials in swine diets. *Prof Anim Sci* 2001;17:217–26.
- Wakabayashi H, Egusa S. *Edwardsiella tarda* (*Paracolobactrum anguillimortiferum*) associated with pond-cultured eel disease. *Bull Jpn Soc Fish* 1973;39:931–6.
- Wang LH, Li XY, Jin LJ, You JS, Zhou Y, Li SY, et al. Characterization of chicken egg yolk immunoglobulins (IgYs) specific for the most prevalent capsular serotypes of mastitis-causing *Staphylococcus aureus*. *Vet Microbiol* 2011;149:415–21.
- Warr GW, Magor KE, Higgins DA. IgY: clues to the origins of modern antibodies. *Immunol Today* 1995;16:392–408.
- Waters JR, Sellwood R. Aspects of genetic resistance to K88 *E. coli* in pigs. The 2nd world congress on genetics applied to livestock production, Madrid; 1982. p. 362.
- Wiedemann V, Linckh E, Kuhlmann R, Schmidt P, Losch U. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. V. *In vivo* studies on protective effects against *Escherichia coli* diarrhea in pigs. *J Vet Med B* 1991;38:283–91.
- Wills FK, Luginbuhl RE. The use of egg yolk for passive immunization of chickens against Newcastle disease. *Avian Dis* 1963;7:5–12.
- Windisch WM, Schedle K, Plitner C, Kroismayr A. Use of phytochemical products as feed additives for swine and poultry. *J Anim Sci* 2008;86(Suppl):E140–8.
- Witteveldt J, Vlask JM, van Hulten MCW. Protection of *Penaeus monodon* against white spot syndrome virus using a WSSV subunit vaccine. *Fish Shellfish Immunol* 2004;16:571–9.
- Wongteerasupaya C, Vicker JE, Sriurairatana S, Nash GL, Akarajamorn A, Tassanakajon A, et al. A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn, *Penaeus monodon*. *Dis Aquat Organ* 1995;21:69–77.
- Wu JL, Nishioka T, Mori K, Nishizawa T, Muroga K. A time-course study on the resistance of *Penaeus japonicus* induced by artificial infection with white spot syndrome virus. *Fish Shellfish Immunol* 2002;13:391–403.
- Yokoyama H, Peralta RC, Diaz R, Sando S, Ikemori Y, Kodama Y. Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic *Escherichia coli* infection in neonatal piglets. *Infect Immun* 1992;60:998–1007.
- Yokoyama H, Peralta RC, Sando S, Ikemori Y, Kodama Y. Detection of passage and absorption of chicken egg yolk immunoglobulins in the gastrointestinal tract of

- pigs by use of enzyme-linked immunosorbent assay and fluorescent antibody testing. *Am J Vet Res* 1993;54:867–72.
- Yokoyama H, Umeda K, Peralta RC, Hashi T, Icatlo Jr FC, Kuroki M, et al. Oral passive immunization against experimental salmonellosis in mice using chicken egg yolk antibodies specific for *Salmonella enteritidis* and *S. typhimurium*. *Vaccine* 1998;16:388–93.
- Yolken RH, Leister F, Wee SB, Miskuff R, Vonderfecht S. Antibodies to rotavirus in chickens' eggs: a potential source of antiviral immunoglobulins suitable for human consumption. *Pediatrics* 1988;81:291–5.
- You JS, Xu YP, He ML, McAllister TA, Thacker PA, Li XY, et al. Protection of mice against enterotoxigenic *E. coli* by immunization with a polyvalent enterotoxin comprising a combination of LTb, STa, and STb. *Appl Microbiol Biotechnol* 2011;89:1885–93.
- You JS. Recombinant polyvalent enterotoxin vaccines against enterotoxigenic *Escherichia coli*. Ph.D. thesis, Dalian University of Technology, China; 2011.
- Zhen YH, Jin LJ, Guo J, Li XY, Li Z, Fang R, et al. Characterization of specific egg yolk immunoglobulin (IgY) against mastitis-causing *Staphylococcus aureus*. *J Appl Microbiol* 2008a;105:1529–35.
- Zhen YH, Jin LJ, Guo J, Li XY, Lu YN, Chen J, et al. Characterization of specific egg yolk immunoglobulin (IgY) against mastitis-causing *Escherichia coli*. *Vet Microbiol* 2008b;130:126–33.
- Zhen YH, Jin LJ, Li XY, Guo J, Li Z, Zhang BJ, et al. Efficacy of specific egg yolk immunoglobulin (IgY) to bovine mastitis caused by *Staphylococcus aureus*. *Vet Microbiol* 2009;133:317–22.