Avian and Poultry Biology Reviews **15** (1), 2004, 25–46

Avian egg antibodies: basic and potential applications

Jennifer Kovacs-Nolan and Yoshinori Mine*
Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT

The existence of an IgG-like molecule in avian eggs, referred to as IgY, has been well documented, and extensive research has been carried out on its characterization, production and purification. Although it is the functional equivalent of mammalian IgG, the major serum antibody found in mammals, IgY is structurally different, and has been found to exhibit several important differences when compared to mammalian antibodies, including its physico-chemical properties and immunological capabilities. Recently, considerable research has focussed on the use of IgY as an alternative to mammalian antibodies for several applications, including for immunotherapeutic applications, especially for the oral passive immunization against various bacteria and viruses. Much research has also been carried out on the use of IgY as a replacement for IgG in various immunodiagnostic and immunoaffinity purification purposes. The use of IgY offers several advantages over polyclonal antibodies produced in mammals, including providing a much more hygienic, cost efficient, convenient, humane and plentiful source of antigen-specific antibodies.

Keywords: avian, egg yolk, antibody, IgY, pathogens, stability, isolation, avian immune system, immunotherapy, diagnostics, affinity chromatography

1. INTRODUCTION

In 1969, Leslie and Clem reported the existence of an immunoglobulin (Ig) G-like molecule in chickens. It was the predominant serum immunoglobulin, or antibody, however its structure was slightly different than that of the mammalian serum antibody IgG, and therefore was termed IgY. It has since been found to be the principal serum antibody of birds, reptiles, and amphibians (Marchalonis, 1977). The chicken immune system has been studied for many years, and these studies have contributed substantially to the understanding of the fundamental concepts of immunology and the development of different immunoglobulin classes (Carlander *et al*., 1999).

Although functionally similar, there are several important differences between mammalian IgG and avian IgY (Sharma, 1997), and the use of avian antibodies offers many advantages over mammalian antibodies. However, only a small fraction of antibodies currently used in laboratories are of avian origin. This could be due to a lack of information or experience with the production and purification techniques involved, or the problems associated with keeping chickens, compared to smaller animals which may more conveniently be housed in a laboratory setting (Hanley *et al*., 1995; Schade *et al*., 1996). The production of specific IgY against many different antigens has been studied, and its application as an immunotherapeutic agent, including its use for the oral passive immunization against enteric pathogens, has been extensively reported. Due to its distinctness from IgG, IgY has also been found to be advantageous in several immunodiagnostic techniques, as well as in immunoaffinity purification, in many cases replacing IgG.

Traditionally, commercially available polyclonal antibodies have been produced in mammals such as mice, rats, rabbits, sheep, goats, and horses, and are generally obtained from sera after immunization of these animals (Schade *et al*., 1996). However, these antibodies cannot be prepared on an industrial scale because of the difficulty in obtaining large quantities of blood, and concerns about animal welfare. The use of hybridoma technology has been used for the preparation of monoclonal antibodies, however it is still far from the successful commercialization of therapeutic monoclonal antibodies due to the expensive cost (Wang and Imanaka, 1995). Bovine colostrum or colostral antibodies have also been examined (Crabb, 1998), however their quantity and antibody specificity have limitations. Some of the real or potential applica-

*To whom correspondence should be addressed: Email: ymine@uoguelph.ca

tions of antibodies, especially for immunotherapeutic purposes, will require kilogram quantities of highly purified antibody, therefore cost-efficient methods of producing large quantities of specific antibodies are required. Recently, the chicken has attracted considerable attention as an alternative source of antibodies. IgY is deposited in the egg yolk in large quantities (Janson *et al*., 1995), and it can be easily purified from the yolk by simple precipitation techniques (Gassmann *et al*., 1990), making chickens an ideal source for specific polyclonal antibodies.

Here we review several aspects of avian immunoglobulins and the avian immune system, including the structure, production and purification of IgY, as well as the many current and potential applications of IgY, especially in the areas of immunotherapy and immunodiagnostics.

2. AVIAN EGG FORMATION

Under modern husbandry conditions, a chicken can lay an average of 250–280 eggs per year. The egg is the largest biological cell which originates from one cell division, and is composed of various important chemical substances for the next generation of birds. An egg is composed of three main parts, the shell, albumen and yolk. The yolk is surrounded by an albumen layer and compartmentalized by an eggshell. The formation of an egg involves the conversion of the feed into egg constituents through a number of intricate and highly coordinated steps as a storehouse of nutrients. The hen normally starts laying at 16–26 weeks of age. The reproductive system of the hen, shown in Figure 1, consists of the ovary and oviduct (Romanoff and Romanoff, 1949). The ovary, which is the site of assembly of the yolk, is a small organ. When the chicken becomes mature (about 150 days old), the ovary has grown to about 7 g, and rapidly increases to about 40 g (around 170 days old) (Epple and Steson, 1980). A mature ovary contains many oocytes, and at least 600–700 of them will become mature yolk. Each oocyte becomes a follicle after being covered with a granular layer. The follicles in the ovary are surrounded by the hen's veins (Burley and Vadehra, 1989). Yolk constituents are synthesized in the liver and they are transported to the follicular walls in the blood. The follicle undergoes a rapid development during which most of the yolk is deposited 6–10 days prior to ovulation, when sufficient yolk has accumulated. The follicle in the ovary is ovulated

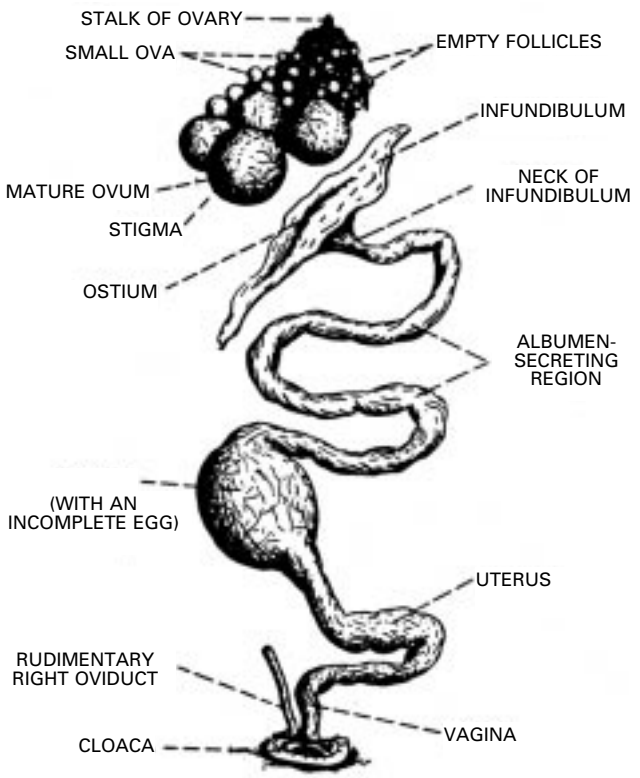

Fig. 1. The reproductive system of the hen: ovary and oviduct. (From Romanoff and Romanoff (1949), reproduced by permission of John Wiley & Sons, Inc., New York).

into the oviduct where the yolk is enveloped in albumen and the shell. It takes 24–27 hours for this development. In laying hens, the oviduct is 40–80 cm long with an average weight 40 g and consists of five regions, infundibulum, magnum, isthmus, uterus and vagina (Burley and Vadehra, 1989). The infundibulum is the top portion of the oviduct, with a broad funnel shaped anterior end (8–9 cm) and a narrow posterior end to receive the ovulated follicles. The ovulated follicle is held for 15–30 minutes, where the yolk probably acquires the outer layer of the vitelline membranes and the chalaza layer of the albumen (Burley and Vadehra, 1989). The albumen-secreting region is the largest part of the oviduct, about 30 cm long and the follicle is held here for 2–3 hours while the egg albumen is secreted to cover the yolk. The isthmus is about 11 cm long and the shell membranes are synthesized here. The egg yolk enveloped with albumen is immediately wrapped by the membrane. The complete synthetic process of the shell formation takes place in the uterus (shell gland) for about 20 hours, while calcium from the blood is deposited to the

shell by assembling a crystalline-like calcium structure on the shell membranes. However, its mechanism still is not well understood. The vagina is the last portion of the oviduct, and the end of the vagina connects with the cloaca. It takes only 5 minutes for the egg to pass through this portion.

Shell eggs consist of about 9.5% shell, 63% albumin, and 27.5% yolk. The total solids content of egg yolk is generally around 50%, but can vary with the age of the hen and the storage of the shell eggs. The major constituents of the solid matter of yolk are proteins and lipids, present mainly in the form of lipoproteins (Li-Chan *et al*., 1995). Their relative amounts can be seen in Table 1. The yolk can be separated by high speed centrifugation into sedimented granules and a clear fluid supernatant called plasma. Granules are composed of 70% α- and β-lipovitellins, 60% phosvitin, and 12% low-density lipoproteins (Burley and Cook, 1961). The plasma is divided into the low-density lipoprotein fraction (33%) and the water soluble fraction (WSF) (5%), which contains the livetins, which are lipid-free globular proteins, including γ-livetin, also referred to as IgY (Li-Chan *et al*., 1995).

Table 1 Chemical composition of egg yolk

Constituent	% (w/v)	Major components (relative %)
Proteins	15.7–16.6	Apovitellenin (I-VI) (37.3%) Lipovitellin apoproteins (40.0%) α-lipovitellin β-lipovitellin Livetins (9.3%) α-livetin (serum albumin) β-livetin (α2 glycoprotein) γ-livetin (γ-globulin) Phosvitin (13.4%) Biotin-binding protein (trace)
Lipids	32.0–35.0	Triglycerol (66%) Phosphatidylcholine (PC) (24%) Phosphatidylethanolamine (PE) (2.8%) Lysophosphatidylcholine (LPC) (0.6%) Sphingomyelin (0.6%) Cholesterol (5.0%) Others (1.0%)
Carbohydrate	0.2–1.0	
Ash	1.1	

Modified from Juneja (1997).

3. AVIAN EGG ANTIBODIES

3.1 Avian immune system

The chicken immune system consists of the Bursa of Fabricius, bone marrow, spleen, thymus, the Harderian gland, lymph nodes, circulating lymphocytes, and various lymphoid tissues. The thymus serves as the primary lymphoid organ for T-cell differentiation, while the antibody-synthesizing B-cells are produced in the Bursa of Fabricius (Sharma, 1997; Carlander *et al*., 1999). The spleen is the centre for plasma cell proliferation and memory B-cells (Carlander *et al*., 1999).

The immune system of vertebrates has the ability to produce an exceedingly high number of different antibody molecules, through the existence of multiple variable (V), diversity (D) and joining (J) elements in the genome (the germ line repertoire), as well as existence of somatic recombination processes and point mutations (Reynaud *et al*., 1985; Parvari *et al*., 1988). However, B-cell formation and generation of diversity are significantly different in the chicken as compared to mammals. The use of such combinatorial diversity is restricted in the chicken as the rearrangement of immunoglobulin genes is not an ongoing process, rather it takes place as a single wave during early embryogenesis. Therefore, the total number of rearrangements from which the chicken B-cell repertoire may be generated is limited to the number of B-cell precursors in the bursa (Reynaud *et al*., 1989, 1991), estimated to be around $2\text{-}3 \times 10^4$ cells (Pink *et al*., 1985). Avian antibodies contain both heavy (H) and light (L) chains that are encoded by two unlinked loci. In the light chain locus there are only single gene segments each for the V and J regions. The heavy chain has only one segment each for V and J regions, and about 15 D segments (Sharma, 1997). Therefore rearrangement contributes little diversity in chicken B-cells, in contrast to mammals, because there are only single gene segments for the V and J regions. Only the D segments serve to introduce a combinatorial factor of diversity (Reynaud *et al*., 1985, 1987, 1989). Birds instead attain antibody diversity using sequences of pseudogenes (25 for the light chain and around 100 for the heavy chain) in a process of gene conversion in which segments of pseudogenes are inserted into the V region (Reynaud *et al*., 1987, 1989; Sharma, 1997). In this way, despite the fact that chickens have an extremely limited number of immunoglobulin genes, compared to mammals, they are capable of producing a wide range of immune responses and diverse antibody molecules (Sharma, 1997).

3.2 Biosynthesis

Three immunoglobulin classes have been shown to exist in the chicken: IgA, IgM, and IgY. The IgA and IgM are similar to mammalian IgA and IgM. Chicken IgY is the functional equivalent of IgG, the major serum antibody found in mammals, and makes up about 75% of the total antibody population (Carlander *et al*., 2000). The serum concentrations of IgY, IgA, and IgM have been reported to be 5.0, 1.25, and 0.61 mg/ml, respectively (Leslie and Martin, 1973). In mammals, the transfer of maternal antibodies can take place after birth, however in the chicken, the maternal antibodies must be transferred to the developing embryo, to give acquired immunity to the chick (Carlander *et al*., 1999; Sim *et al*., 2000). Antibody, specifically IgA and IgM, is secreted into the ripening egg follicle and is incorporated into the egg white in the oviduct along with the egg albumen secretion. Serum IgY is selectively transferred to the yolk via a receptor on the surface of the yolk membrane which is specific for IgY translocation (Loeken and Roth, 1983; Tressler and Roth, 1987; Morrison *et al*., 2002). Egg white contains IgA and IgM at concentrations of around 0.15 and 0.7 mg/ml, respectively, whereas the yolk may contain from 5 to 25 mg/ml of IgY (Rose *et al*., 1974; Schade *et al*., 1991; Li *et al*., 1997). Mammalian equivalents of IgE and IgD have not been identified in chickens (Sharma, 1997).

3.3 Structure of immunoglobulin Y

Although similar in function, the structure of IgY is significantly different than that of mammalian IgG (Carlander *et al*., 1999) (Figure 2). IgY contains two heavy (H) and two light (L) chains and has a molecular mass of 180 kDa, larger than that of mammalian IgG (159 kDa). IgY possesses a larger molecular weight H chain (68 kDa) as compared to that from mammals (50 kDa). The H chain of IgG consists of four domains: the variable domain (V_H) and three constant domains (Cγ1, Cγ2 and Cγ3). The Cγ1 domain is separated from Cγ2 by a hinge region, which gives considerable flexibility to the Fab fragments. In contrast, the H chain of IgY does not have a hinge region, and possesses four constant domains (Cυ1–Cυ4) in addition to the variable domain. Sequence comparisons between IgG and IgY have shown that the Cυ2 and Cυ3 domains of IgG are closely related to the Cυ3 and Cυ4 domains, respectively, of IgY, while the equivalent of the Cυ2 domain is absent in the IgG chain, having been replaced by the hinge region (Warr *et al*., 1995). The content of β-sheet structure in the constant domains of IgY has been reported to be lower than that of IgG, and the flexibility between the Cυ1 and Cυ2 domains, corresponding to the hinge region of IgG, is less than that of IgG (Shimizu *et al*., 1992). Unlike IgG, IgY has two additional Cys residues, Cys331 and Cys 338, in the Cυ2–Cυ3 junction, which likely participate in the inter-υ chain disulfide linkages (Warr *et al*., 1995).

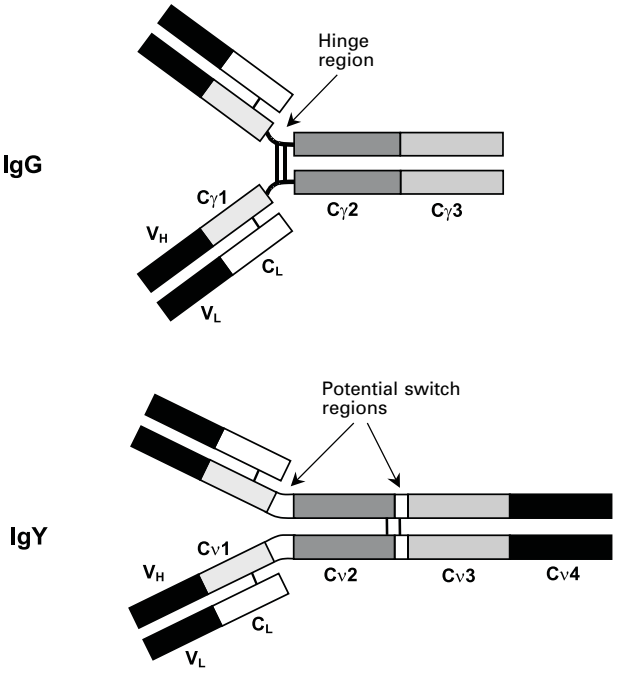

Fig. 2. Structure of IgG and IgY. Disulfide bonds are indicated by lines connecting the two chains (Adapted from Warr *et al*. (1995)).

Both IgG and IgY are known to contain Asn-linked oligosaccharides, however the structure of oligosaccharides in IgY differ from those of any mammalian IgG, containing unusual monoglucosylated oligomannose type oligosaccharides with $Glc_1Man_{7-9}GlcNAc_2$ structure (Ohta *et al*., 1991; Matsuura *et al*., 1993).

Furthermore, the isoelectric point of IgY is lower than that of IgG (Polson *et al*., 1980a), IgY does not associate with mammalian complement, and the binding of IgY with human and bacterial Fc-receptors on cell surfaces is less than that of IgG (Gardner and Kaya, 1982). IgY does not bind to *Staphylococcus* protein A or *Streptococcus* protein G (Kronvall *et al*., 1974; Carlander *et al*., 1999) or rheumatoid factors (RF) (Larsson and Sjöquist, 1988) as does IgG. The

differences between IgG and IgY are summarized in Table 2.

3.4 Origins of immunoglobulin Y

Although IgM is the only universally distributed antibody, and is therefore believed to be the precursor for all immunoglobulin classes, current evidence suggests that IgY may have instead been the immediate progenitor of both IgG and IgE (Warr *et al*., 1995). IgY possesses the both the properties of IgG and IgE, in that it is the major serum antibody, similar to IgG, and it possesses the ability to mediate anaphylactic reactions, like IgE. The cloning and sequencing of the genes encoding the H and L chains of IgY has been carried out, and the structure of these polypeptides has been determined (Reynaud *et al*., 1983; Parvari *et al*., 1988). The similarity of IgY to IgE is apparent in terms of the number of C_H domains, and the number and organization of intradomain and interchain disulfide bonds. As well, amino acid sequence data supports an evolutionary hierarchy in which IgE and IgG may have arisen from IgY (Warr *et al*., 1995). The properties of IgY, IgG, and IgE can be seen in Table 3. It has been suggested that the functions of IgY may have been maladaptive, and therefore through evolution gave rise to the superior IgG. Compared to IgG, IgY has limited diversity and affinity maturation. It also does not have the ability to precipitate or agglutinate multivalent antigens, unless at high salt concentrations (around 1.5 M) (Hersh and Benedict, 1966), perhaps due to steric hindrance caused by the closely aligned Fab arms of the IgY molecule. High salt concentrations may serve to release the Fab arms, permitting agglutination. This would support the theory that the IgG hinge region arose from the condensation of the C_H2 domain of the IgY, conferring additional flexibility and functional diversity on the IgG (Warr *et al*., 1995).

Table 2 Comparison of mammalian IgG and chicken IgY

Animals	Rabbit (IgG)	Chicken (IgY)
Source of antibody	Blood serum	Egg yolk
Kind of antibody	Polyclonal	Polyclonal
Antibody sampling	Bleeding	Collecting eggs
Antibody amount	200 mg/bleed (40 ml blood)	100–150 mg/egg
Quantity of antibody (per year)	1400 mg	40 000 mg
Amount of specific antibody	~5%	2–10%
Protein A/G binding	Yes	No
Interaction with mammalian IgG	Yes	No
Interaction with rheumatoid factor	Yes	No
Activation of mammalian complement	Yes	No

Based on Gottstein and Hemmeler (1985) and Schade *et al*. (1991).

Table 3 Similarities in structure and functional properties between IgY and both IgG and IgE

	IgG	IgY	IgE
Molecular weight (kDa)	150	180	200
Weight of H chain (kDa)	50	68	75
Number of C domains	3	4	4
Hinge region	Yes	No	No
Percent (%) of total Ig	80	75	0.002
Carbohydrate content	2.5–4%	~4%[a]	11.7%
Stable at high temperatures (> 65° C)	Yes	No	No
Activation of complement	Yes	No	No
Binding to Fc receptors	Yes	No	No
Reactivity with Protein A	Yes	No	No

Adapted from Barrett (1983), Benjamini *et al*. (1996), and Janeway and Travers (1996).
[a]From Ohta *et al*. (1991).

3.5 Production and purification of immunoglobulin Y

Chicken eggs present an ideal alternative antibody source to mammals, as the IgY in the chickens' blood is transported to the egg and accumulates in the egg yolk in large quantities. Hens usually lay about 280 eggs in a year. Egg yolk contains a considerable amount of IgY, around 100–150 mg/egg (Rose *et al*., 1974). Therefore, an immunized hen yields more than 40 g of IgY a year through eggs, equivalent to that from 40 rabbits. Jensenius *et al*. (1981) reported that IgY corresponding to almost half a liter of serum may be recovered from a chicken in one month. This is 5–10 times higher than that from the blood of a rabbit. Over a period of slightly less than 6 weeks, 298 mg of specific IgY against *Echino coccusgranulosus* was obtained from eggs, compared to only 16.6 mg from the rabbit's blood, 18 times more from yolk (Gottstein and Hemmeler, 1985).

Separation of IgY from egg yolk has been extensively studied. Egg yolk is a fluid emulsion with a continuous phase of lipoprotein particles. Egg yolk

lipids therefore exist as lipoproteins (Burley and Cook, 1961). The major problem in isolating IgY from egg yolk is separating the lipoproteins from egg yolk prior to purification of the IgY. Based on this strategy, many purification methods of IgY have been reported, and are summarized in Table 4. Several methods have been reported using water dilution, followed by centrifugation or ultrafiltration, to isolate the water soluble fraction (WSF) (Akita and Nakai, 1992; Kim and Nakai, 1996, 1998). This method relies on the aggregation of yolk lipoproteins at low ionic strengths, as reported by Jensenius *et al*. (1981). Likewise, freezing and thawing of diluted yolk, which results in the formation of lipid aggregates that are large enough to be removed by conventional low speed centrifugation (Jensenius and Koch, 1993), has also been employed, often as a preliminary purification step, and was recently reported by Deignan *et al*. (2000) to result in a purity of around 70%. For these dilution methods, pH and extent of dilution are very important for optimal IgY recovery, and Nakai *et al*. (1994) found that the best results were obtained using a six-fold water dilution, at pH 5.0. Similar to IgG purification, ammonium sulfate precipitation has also been reported for the purification of IgY from WSF, following lipoprotein precipitation (Akita and Nakai, 1992; Svendsen *et al*., 1995). Other methods of IgY separation include: lipoprotein separation by ultracentrifugation (McBee and Cotterill, 1979), delipidation by organic solvents (Bade and Stegemann, 1984; Polson *et al*., 1985; Hatta *et al*., 1988; Polson, 1990; Kwan *et al*., 1991; Horikoshi *et al*., 1993; McLaren *et al*., 1994; Svendsen *et al*., 1995), lipoprotein precipitation by polyethylene glycol (Polson *et al*., 1980b, Akita and Nakai, 1993; Svendsen *et al*., 1995), sodium dextran sulfate (Jensenius *et al*., 1981; Akita and Nakai, 1993), and dextran blue (Bizhanov and Vyshniausskis, 2000), and natural gums such as xanthan gum (Akita and Nakai, 1993a) and sodium alginate (Hatta *et al*., 1990). Chang *et al*. (2000) recently reported the precipitation of over 90% of lipoproteins from yolk using λ-carrageenan, sodium alginate, carboxymethyl cellulose, and pectin. Ion exchange chromatography has also been reported as a final step in IgY purification (McCannel and Nakai, 1990; Akita and Nakai, 1992; Fichtali *et al*., 1992, 1993), as well as hydrophobic interaction chromatography (Hassl and Aspock, 1988).

Because of the failure of IgY to bind proteins A and G, and its sensitivity to traditional affinity purification conditions, several other methods of affinity chromatography have been examined for the purification of IgY, including immobilized metal ion affinity chromatography (McCannel and Nakai, 1989; Greene and Holt, 1997), thiophilic interaction chromatography (Hansen *et al*., 1998), affinity chromatography using alkaline conditions (Kuronen *et al*., 1997), and synthetic peptide ligands, designed specifically for immobilizing antibodies (Fassina *et al*., 1998; Verdoliva *et al*., 2000). As well, Erhard *et al*. (1996) described a method for the purification of mouse IgG subclass specific IgY using indirect affinity chromatography with protein G Sepharose.

Table 4 Methods of purifiying IgY

Purification method	Reference
Ultrafiltration	Akita and Nakai, 1992 Kim and Nakai, 1996, 1998
Precipitation and extraction	
Polyethylene glycol (PEG)	Polson *et al*., 1980 Akita and Nakai, 1993
Dextran sulphate	Jensenius *et al*., 1981 Akita and Nakai, 1993
Ethanol	Polson *et al*., 1985 Hatta *et al*., 1988 Horikoshi *et al*., 1993 Kwan *et al*., 1991
Ammonium sulfate	Akita and Nakai, 1992 Svendsen *et al*., 1995
Chloroform	Polson, 1990
Dextran blue	Bizhanovv and Vyshniausskis, 2000
Caprylic acid	McLaren *et al*., 1994 Svendsen *et al*., 1995
Propanol	Bade and Stegemann, 1984
Natural gums	
k-carrageenan	Hatta *et al*., 1990 Chang *et al*., 2000
Sodium alginate	Hatta *et al*., 1988 Chang *et al*., 2000
Xanthan gum	Akita and Nakai, 1993
Carboxymethylcellulose (CMC)	Chang *et al*., 2000
Pectin	Chang *et al*., 2000
Chromatography	
Ion exchange chromatograpy	McCannel and Nakai, 1990 Akita and Nakai, 1992 Fichtali *et al*., 1992, 1993
Affinity chromatography	McCannel and Nakai, 1989 Verdoliva *et al*., 2000 Greene and Holt, 1997 Hansen *et al*., 1998 Fassina *et al*., 1998
Hydrophobic interaction chromatography	Hassl and Aspock, 1988

Due to the prevalence of individuals with allergies to egg proteins, the presence of extraneous egg proteins in IgY preparations designed for food or nutraceutical use must be taken into consideration, and the development of thorough purification procedures is therefore of utmost importance. Akita and Nakai (1993b) also suggested the use of enzymatic digestion of IgY, to remove the Fc portion of the immunoglobulin molecule, which is considered the most allergenic portion, in order to further reduce the risk of an allergic response to IgY preparations.

3.6 Physico-chemical properties

IgY and IgG differ not only in structure, but also in their stability to pH, heat, and proteolytic enzymes. Although the stability of both immunoglobulins was similar when subjected to alkaline conditions, IgY showed much less stability than that of rabbit IgG to acid denaturation. Shimizu *et al*. (1992, 1993) found that the activity of IgY was decreased by incubating at pH 3.5 or lower, and completely lost at pH 3. The rabbit IgG antibodies, on the other hand, did not demonstrate a loss of activity unit the pH was decreased to 2, and even then some activity still remained. Similar results were also observed by Hatta *et al*. (1993), using IgY produced against human rotavirus. Similarly, the IgY was significantly more sensitive to heating than the rabbit IgG. Shimizu *et al*. (1992) found that the activity of IgY was decreased by heating for 15 minutes at 70° C or higher, whereas that of the IgG did not decrease until 75–80° C or higher. Hatta *et al*. (1993) found, using differential scanning calorimetry (DSC), that the temperature corresponding to the maximum of denaturation endotherm (T_{max}) was 73.9° C for IgY and 77.0° C for IgG. Shimizu *et al*. (1994), however, described the addition of sugar to an IgY solution, and found high concentrations of sugar allowed the IgY to maintain activity when subjected to high heat (75–80° C), low pH (3), or high pressure (5000 kg/cm^2).

IgY, like IgG, has been found to be relatively resistant to trypsin and chymotrypsin digestion, but sensitive to pepsin digestion (Shimizu *et al*., 1988). Hatta *et al*. (1993) found that almost all of the IgY activity was lost following digestion with pepsin, however activity remained even after 8 hours incubation with trypsin or chymotrypsin. Otani *et al*. (1991) found that IgY was, however, more susceptible to digestion with trypsin, chymotrypsin and pepsin than IgG. The proteolytic digestion of antibodies is a common technique, used to remove the cross-reacting Fc portion of the antibody molecule. Akita and Nakai (1993b) noted further differences between IgY and IgG, with the peptic digestion of IgY resulting in mainly monovalent Fab' fragments, while the peptic digestion of IgG yields the bivalent F(ab')$_2$ fragments.

The structural factors resulting in the stability differences of the two immunoglobulins are unknown, as immunoglobulins are large, complicated molecules, composed of heterogeneous polypeptides. Shimizu *et al*. (1992) predicted that the lower content of β-structure in IgY may indicate that the conformation of IgY is more disordered and therefore less stable than mammalian IgG. The lack of a hinge region in IgY could be another factor affecting molecular stability. The lower flexibility of the Cν1 and Cν2 domains of IgY, as compared to the hinge region of IgG, may cause the rapid inactivation of the antibody by the various treatments, because the flexibility of the hinge region is considered to influence the overall properties of immunoglobulin molecules (Pilz *et al*., 1977).

3.7 Advantages of immunoglobulin Y

The use of chickens for the production of polyclonal antibodies provides several advantages over the traditional method of producing antibodies in mammals. In contrast to mammalian serum, egg yolk contains only the single class of antibody, IgY, which can be easily purified from the yolk by simple precipitation techniques (Gassmann *et al*., 1990). The phylogenetic distance between chickens and mammals renders possible the production of antibodies, in chickens, against highly conserved mammalian proteins, that would otherwise not be possible in mammals, and much less antigen is required to produce an efficient immune response (Larsson *et al*., 1988). Chicken antibodies will also recognize different epitopes than mammalian antibodies, giving access to a different antibody repertoire than with mammalian antibodies (Carlander *et al*., 1999). As well, the method of producing antibodies in hens is much less invasive, requiring only the collection of eggs, rather than the collection of blood, and is therefore less stressful on the animal (Schade *et al*., 1991), and sustained high titres in chickens reduce the need for frequent injections (Gassmann *et al*., 1990). The animal care costs are also lower for the chicken compared to that for

mammals, such as rabbits (Carlander *et al*., 2000). Hens therefore provide a more hygienic, cost efficient, convenient, and plentiful source of antibodies, as compared to the traditional method of obtaining antibodies from mammalian serum (Gassmann *et al*., 1990; Carlander *et al*., 2000). Nakai *et al*. (1994) estimated that the productivity of antibodies in hens is nearly 18 times greater than that by rabbits based on the weight of antibody produced per head. Because of the high yolk IgY concentrations, over 100 mg of IgY can be obtained from one egg (Akita and Nakai, 1992). A laying hen produces approximately 20 eggs per month, therefore over 2 g of IgY per month may be obtained from a single chicken (Carlander *et al*., 1999). In the egg, IgY is stable for months, and once purified it may be stored for years in the cold (Larsson *et al*., 1993). As the industrial scale automated collection and separation of eggs is currently carried out, the large-scale production of specific IgY for immunotherapeutic purposes is feasible (Cotterill and McBee, 1995). Similarly, vaccination of chicken flocks has long been used to control avian infections (Sharma, 1999), making the injection of chickens required for large-scale antibody production also feasible.

4. APPLICATIONS OF IMMUNOGLOBULIN Y

4.1 Immunotherapeutic applications of IgY

Although it is a recent concept in human medicine, passive immunization using specific antibodies has been studied extensively in animals, and presents an attractive approach to establish passive immunity against pathogens in both humans and animals (Carlander *et al*., 2000). In the past, immunotherapy was carried out via the systemic or intravenous administration of specific antibodies, for such applications as a targeting agent for cancer diagnosis and therapy, the inactivation of toxic substances including drugs, and as passive immunotherapy for neoplastic or infectious diseases (Reilly *et al*., 1997). However, there has been increasing interest in the oral administration of specific antibodies for localized treatment of infections (Reilly *et al*., 1997). The increase in antibiotic-resistant bacteria (Crabb, 1998; Wierup, 2000), and the desire to treat pathogens that do not respond to antibiotics (Carlander *et al*., 2000l), such as viral pathogens, along with the escalating number of immunocompromised individuals (Casadeval and Scharff, 1995) has prompted much research into the administration of specific antibodies as an alternative to antibiotics and antimicrobial chemotherapy to treat infections. It is for this reason that much of the IgY research carried out has been with regard to immunotherapy. The potential applications of IgY for the prevention and treatment of infections caused by pathogenic bacteria and viruses have been studied at length (Table 5), and are discussed below.

Human rotavirus (HRV) has been identified as the major causative agent of acute infantile gastroenteritis (Yolken *et al*., 1988), infecting up to 90% of children under the age of three and resulting in more than a million deaths annually (Prasad *et al*., 1990; White and Fenner, 1994). Characteristically localized to the epithelial cells of the gastrointestinal tract, HRV causes a shortening and atrophy of the villi of the small intestine (Kapikian and Chanock, 1996), resulting in decreased water absorption, leading to severe diarrhoea and vomiting, and eventually death due to dehydration (Ludert *et al*., 1996; Hochwald and Kivela., 1999). Yolken *et al*. (1988) found that the oral administration of antibodies isolated from the eggs of chickens immunized with three different serotypes of rotavirus (mouse, human and monkey) were capable of preventing rotavirus-induced diarrhoea in mice infected with murine rotavirus, whereas IgY isolated from the eggs of unimmunized chickens failed to prevent rotavirus infection. Using an HRV infection model in suckling mice, Hatta *et al*. (1993) reported that anti-HRV IgY decreased the incidence of rotavirus-induced diarrhoea in mice, both when administered before and after HRV challenge, suggesting its use for both therapeutic and prophylactic applications. Similarly, Ebina (1996) also observed the prevention of HRV-induced symptoms in mice using anti-HRV IgY. Therefore, anti-HRV IgY has the potential to significantly reduce the morbidity and mortality associated with HRV infection. Recently the production of specific IgY against recombinant HRV coat protein VP8*, a cleavage product of the rotavirus spike protein, VP4, was reported (Kovacs-Nolan *et al*., 2001). VP4 has been implicated in several important functions, including cell attachment and penetration, hemagglutination, neutralization, and virulence (Mackow *et al*., 1988; Both *et al*., 1994; Desselberger and McCrae, 1994; Nejmeddine *et al*., 2000), and VP8 has been found to play a significant role in viral infectivity and neutralization of the virus. Using only VP8 should result in highly specific

Table 5 Specific IgY against various bacterial and viral pathogens, and the species targeted for immunotherapy

Pathogen	Target Species	Effect of IgY	Refs
Rotavirus	Cow	Protecting calves from bovine rotavirus (BRV)-induced diarrhea	Kuroki *et al*., 1994
	Human	Preventing murine rotavirus in mice	Yolken *et al*., 1988
	Human	Preventing human rotavirus (HRV)-induced gastroenteritis in mice	Ebina *et al*., 1996
	Human	Prevention and treatment of HRV-induced gastroenteritis using murine model	Hatta *et al*., 1993
	Human	Prevention of HRV infection *in vitro*, using IgY against recombinant coat protein VP8*	Kovacs-Nolan *et al*., 2001
Coronavirus	Cow	Protection of neonatal calves from bovine coronavirus (BCV)-induced diarrhea	Ikemori *et al*., 1997
Escherichia coli	Pig	Preventing K88 +, K99 +, 987P + ETEC infection in neonatal piglets	Yokoyama *et al*., 1992
	Cow	Protecting neonatal calves from fatal enteric colibacillosis by K99-piliated ETEC	Ikemori *et al*., 1992
	Pig	Inhibiting adhesion of ETEC K88 to piglet intestinal mucosa	Jin *et al*., 1998
	Pig	Prevention of ETEC K88+ infection in neonatal and early weaned piglets	Marquardt *et al*., 1999
	Pig	Inhibiting shedding of F18+ *E. coli* in infected piglets	Imberechts *et al*., 1997
	Human	Preventing diarrhea in rabbits challenged with ETEC	O'Farrelly *et al*., 1992
Salmonella	Human	Protecting mice challenged with *S. enteriditis* or *S. typhimurium* from experimental salmonellosis	Yokoyama *et al*., 1998a
	Cow	Preventing fatal salmonellosis in neonatal calves exposed to *S. typhimurium* or *S. dublin*	Yokoyama *et al*., 1998b
	Human	Inhibiting adhesion of *S. enteriditis* to human intestinal cells	Sugita-Konishi *et al*., 2000
	Human	Preventing mice challenged with with *S. enteriditis* from experimental salmonellosis	Peralta *et al*., 1994
Yersinia	Fish	Protection of rainbow trout against *Y. ruckeri* infection	Lee *et al*., 2000
Edwardsiella	Fish	Preventing Edwardsiellosis of Japanese eels infected with *Edwardsiella tarda*	Hatta *et al*., 1994
IBDV	Chicken	Protecting chicks from infectious bursal disease virus	Eterradossi *et al*., 1997
Staphylococcus	Human	Inhibiting the production of *Staphylococcus aureus* enterotoxin-A	Sugita-Konishi *et al*., 1996
Pseudomonas	Human	Inhibiting the growth of *Pseudomonas aeruginosa*	Sugita-Konishi *et al*., 1996
PEDV	Pig	Protection of piglets against porcine epidemic diarrhea virus	Kweon *et al*., 2000
Streptococcus	Human	Prevention of *S. mutans* adhesion *in vitro* and *in vivo*	Hatta *et al*., 1997a
	Human	Prevention of *S. mutans* accumulation and reduction of caries in rats using IgY against *S. mutans*. GBP	Smith *et al*., 2001
	Human	Reduction of caries development in animal model	Otake *et al*., 1991

antibodies, capable of neutralizing the virus. Kovacs-Nolan *et al*. (2001) immunized chickens with recombinant VP8*, and found that the resulting anti-VP8* IgY exhibited significant neutralizing activity, *in vitro*, against the Wa strain of HRV, indicating that anti-VP8* IgY may be used for the prevention and treatment of HRV infection.

Neonatal calf diarrhoea, caused by bovine rotavirus (BRV), is a common disease, and significant cause of mortality (Lee *et al*., 1995), in cattle. The passive protection of calves against BRV infection, using anti-BRV IgY, has also been demonstrated (Kuroki *et al*., 1994).

Similar to BRV, bovine coronavirus (BCV) is an important cause of neonatal calf diarrhoea and acute diarrhoea in adult cattle, however BCV may be more severe as it multiplies in both the small and large intestines (Kapil *et al*., 1990). Ikemori *et al*. (1997) examined the protective effect of anti-BCV antibodies in egg yolk and colostrum in calves challenged with BCV. They found that control calves which received no antibodies experienced severe diarrhoea and all died within 6 days after infection, whereas the calves fed milk containing egg yolk or colostrums all survived and had positive weight gain. The results indicated that orally administered egg yolk or colostral antibodies were capable of passively protecting calves against BCV infection, with the egg yolk antibodies providing a higher degree of protection, and therefore offering a more efficacious alternative to existing methods of passive protection against BCV.

Diarrhoea due to enterotoxigenic *Escherichia coli* (ETEC) is a major health problem in humans and animals. ETEC is the most common cause of enteric colibaccilosis encountered in neonatal calves (Moon *et al*., 1976), piglets (Morris and Sojka, 1985) and children in developing countries and travellers to these countries (Sack, 1986). It accounts for one billion diarrhoeal episodes annually and perhaps one million deaths each year (Sack, 1986). One half of all travellers to developing countries also develop diarrhoea (Svennerholm *et al*., 1989). It also causes significant economic losses to the pig industry from both mortality and reduced growth rates, killing 1.5–2.0% pigs weaned (Hampson, 1994). The strains of ETEC which are associated with intestinal colonization and cause severe diarrhoea are the K88, K99 and 987P fimbrial adhesins (Parry and Rooke, 1985). It has been reported that feeding colostrum from vaccinated cows prevented diarrhoea due to infectious *E. coli* in infants (Hilpert *et al*., 1977). Milk IgG has been used as an effective prophylactic against travellers' diarrhoea (Tacket *et al*., 1988). IgY could be an alternative source of immunoglobulins for the prevention of ETEC infection, as it has been found to inhibit the binding of *E. coli* to the intestinal musosa (Jin *et al*., 1998). IgY raised against ETEC antigen has been administered orally to piglets and has offered a potential prophylactic and therapeutic approach for controlling ETEC-induced diarrhoea (Marquardt *et al*., 1999). Marquardt *et al*. (1999) found that the IgY titre was much higher when *E.coli* fimbrial antigen was used, rather than the whole cell (Marquart *et al*., 1999). Imberechts *et al*. (1997) raised IgY against *E.coli* F18ac fimbriae, and *in vitro* adhesion tests demonstrated that the IgY inhibited attachment of F18ac positive *E. coli* to the intestinal mucosa. The anti-F18ab antibodies were also found to diminish cases of diarrhoea and death in animals infected with F18ac positive *E. coli*. Yokoyama *et al*. (1992) studied the passive protective effect of IgY against ETEC infection in neonatal piglets. Orally administered IgY was found to protect in a dose dependent manner against infection with each of the three strains of *E. coli* in passive immunization trials. They also demonstrated that *E. coli* K88, K99 and 987P strains adhered equally to porcine duodenal and ileal epithelial cells but failed to so in the presence of homologous anti-fimbrial IgY (Yokoyama *et al*., 1992a; Ikemori *et al*., 1993). In another animal feeding study, 21 day old pigs were challenged with a dose of the ETEC (10^{12} cfu). IgY was administered to the piglets in milk three times a day for 2 days. Control piglets developed severe diarrhoea within 12 hours and 30% of the pigs died. In contrast, the pigs given IgY exhibited no sign of diarrhoea 24 or 48 hours after treatment (Marquardt *et al*., 1999). The passive protective effect of anti-ETEC IgY, in neonatal calves, against fatal enteric collibacillosis, has also been studied (Ikemori *et al*., 1992). Calves fed milk containing IgY had transient diarrhoea, 100% survival and good body weight gain during the course of the study. O'Farrelly *et al*. (1992) also reported the prevention of ETEC in rabbits, through the oral administration of anti-ETEC IgY. Because the oral administration of anti-ETEC IgY has proven to be successful for the treatment of gastrointestinal infections of animals, the clinical application of passive immunization of IgY against diarrhoea is now being examined, to prevent and treat ETEC infection in infants.

Salmonella spp. are a significant cause of food poisoning. It is estimated that 2–4 million cases of salmonellosis occur in the USA annually (Bell and Kriakides, 1998). Symptoms include fever, abdominal pain, headache, malaise, lethargy, skin rash, constipation and changes in mental state. The elderly, infants and those with impaired immune systems may develop more severe symptoms. In these cases, the infection may spread from the intestines to the blood stream and then to other sites in the body, and can cause death. *Salmonella enteritidis* (SE) and *Salmonella typhimurium* (ST), in particular, are the major agents of food poisoning (Bell and Kriakides, 1998). Salmonella has a variety of surface components which are virulence related, including outer membrane protein (OMP) (Isibasi *et al*., 1988; Udhayakumar and Muthukkaruppan, 1987), lipopolysaccharides (LPS) (Sunwoo *et al*., 1996; Mine, 1997), flagella (Fla), and in some strains, fimbrial antigen (Thorns *et al*., 1990; Thorns *et al*., 1992). OMP plays a role in pathogenicity determination (Galdiero *et al*., 1990), and has been used successfully for vaccine applications, in both active and passive immunization studies (Isibasi *et al*., 1988; Udhayakumar and Muthukkaruppan, 1987). LPS was also shown to elicit a strong immunogenic reaction, producing large amounts of LPS-specific IgY, and has shown potential application for the inhibition of salmonella adhesion and prevention of disease (Sunwoo *et al*., 1996; Mine, 1997). The passive protective efficacy of chicken IgY specific for OMP, LPS or flagella (Fla) in controlling experimental salmonellosis in mice was examined. In mice challenged with SE (2×10^9 cfu), antibody treatment resulted in a survival rate of 80%, 47% and 60% using OMP, LPS or Fla-specifc IgY, respectively, in contrast to only 20% in control mice. In case of ST (2×10^7), the survival rate was 40%, 30% and 20% using OMP, LPS or Fla specific IgY, while it was 0% in control mice (Yokoyama, *et al*., 1998a). A novel fimbrial antigen, SEF14, produced mainly by SE and *S. dublin* strains, was described by Peralta *et al*. (1994). Mice challenged with SE and treated with anti-SEF14 IgY had a survival rate of 77.8%, compared to a 32% survival rate in the control mice, fed normal egg yolk IgY. Salmonella infection in calves is also a worldwide problem, and two serovars, ST and *S. dublin* account for most salmonellosis within the first 2 weeks after birth. Passive protection against ST and *S. dublin* was investigated by orally inoculating calves with SE or *S. dublin*, and administering IgY against SE or *S. dublin* orally 3 times a day for 7 to 10 days after challenge exposure. All control calves died within 7–10 days, whereas low titer IgY treated calves had 60–70% mortality, and only fever and diarrhoea, but not death, were observed in calves given the higher titer IgY (Yokoyama *et al*., 1998b). IgY has also been found to inhibit the adhesion of SE to human intestinal cells, *in vitro* (Sugita-Konishi *et al*., 1996, 2000). These results demonstrate that IgY specific for *Salmonella spp*. is protective against fatal salmonellosis, and may be clinically useful during a salmonellosis outbreak.

The passive immunization of rainbow trout (*Oncorhynchus mykiss*) against infection with *Y. ruckeri* using IgY has been studied (Lee *et al*., 2000). The *Y. ruckeri* is the causative agent of enteric redmouth disease, a systemic bacterial septicemia of salmonid fish (Stevenson *et al*., 1993). Persistence of *Y. ruckeri* for long periods in carrier fish and shedding of bacteria in feces can present a continuing source of infection. If a population of carrier fish could be substantially cleared by oral administration of anti-*Y. ruckeri* antibody treatments, it may be a cost-effective alternative to slaughtering a stock of fish which pose a health risk. Groups of rainbow trout that had been fed anti-*Y. ruckeri* IgY 2 hours prior to an immersion challenge with *Y. ruckeri* had lower mortalities after 8 days compared with fish fed with normal food before the challenge. The group fed IgY appeared to have a lower number of infected fish after 8 days, based on organ and intestine culture. In a subsequent trial of the feeding procedures with triplicate replicates of the groups, the IgY-fed group showed lower mortalities than groups receiving normal feed (Lee *et al*., 2000). The numbers of IgY-fed fish carrying *Y. ruckeri* in intestine samples appeared lower than the normal-feed controls, regardless of whether the feeding was given before or after the challenge. The oral administration of specific IgY against fish pathogens with feed would provide an alternative to methods using antibiotics and chemotherapy for prevention of fish diseases in fish farms. Moreover, the oral feeding of active IgY would be a novel approach for preventing viral infection diseases of fish because no medicine has been reported to be effective.

Edwardsiella tarda is another important fish pathogen, which is spread by infection through the intestinal mucosa. Edwardsiellosis in Japanese eels is a serious problem for the eel farming industry, and egg

yolk antibodies have been investigated for the prevention of this infectious disease, as treatment with antibiotics has been found to promote the growth of bacterial-resistant strains (Hatta *et al*., 1994). Eels were challenged with *E. tarda* (10^5–10^6 cfu), and anti-*E. tarda* was then orally administered. The infected eels died within 15 days, whereas the eels given IgY survived without any symptoms of *E. tarda* infection, suggesting that the orally administered anti-*E. tarda* IgY may provide an effective approach to prevent *E. tarda* infection in eels (Hatta *et al*., 1994; Gutierrez *et al*., 1993).

Streptococcus mutans serotype c is thought to be the principal causative bacterium of dental caries in humans. The molecular pathogenesis of *S. mutans*-associated dental caries involves a series of binding events that eventually lead to the accumulation of sufficient numbers of these cariogenic bacteria to cause disease (Hamada and Slade, 1980). Chicken antibodies against *S. mutans* MT8148 serotype c or cell-associated glucosyltransferase were prepared and tested against dental caries (Otake *et al*., 1991; Hamada *et al*., 1991; Chang *et al*., 1999). Consumption of a cariogenic diet containing more than 2% IgY-containing yolk powder resulted in significantly lower caries scores (Otake *et al*., 1991), and effective passive protection for the prevention of colonization of *S. mutans* in the oral cavity. It has also been reported that mouth rinse containing IgY specific to *S. mutans* was effective in preventing the dental plaque of humans *in vitro* and *in vivo* (Hatta *et al*., 1997a). Recently, Smith *et al*. (2001) produced IgY against the glucan binding protein B (GBP-B) of *S. mutans*. GBPs are believed to be involved in *S. mutans* biofilm development, and antibodies against GBP-B appear to have the potential to modulate infection and disease caused by *S. mutans* (Smith *et al*., 2001). Using a rat model of dental caries, they found that those rats treated with anti-GBP-B IgY displayed a decrease in *S. mutans* accumulation, as well as a decrease in the overall amount of dental caries, as compared to control rats. These studies indicate that IgY against *S. mutans*, or its components, may act to interfere with *S. mutans* accumulation, and control plaque and the subsequent oral health problems associated with plaque accumulation.

In addition, specific IgY has been shown to be effective at preventing and treating several other pathogens. Sugita-Konishi *et al*. (1996) found that specific IgY was capable of preventing the pathogenesis of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Its use has also been suggested for the passive protection of chicks against infectious bursal disease virus (IBDV) (Eterradossi *et al*., 1997), and for the protection against porcine epidemic virus (PEDV) in piglets (Kweon *et al*., 2000).

4.2 Diagnostic applications of immunoglobulin Y

In addition to their therapeutic importance, polyclonal antibodies are of great importance in biological and medical research, where they serve as essential components in a variety of diagnostic systems used for the qualitative and quantitative determination of a wide range of substances (Schade *et al*., 1996). Polyclonal IgG has long been the antibody of choice for such diagnostic applications, likely due to tradition (Larsson *et al*., 1993). The antigen-binding specificity of IgY is comparable to IgG, and they both can detect antigens with high specificity (Hatta *et al*., 1997b), however, IgY presents several advantages over IgG. As mentioned previously, due to evolutionary differences, the use of chickens for antibody production allows the production of antibodies against conserved mammalian proteins, and can result in an enhanced immune response not possible in mammals. It has also been suggested that, for this reason, chicken antibodies will bind to more epitopes on a mammalian protein, resulting in an amplification of signal in immunological assays (Olovsson and Larsson, 1993), as well as a diversified antibody repertoire (Carlander *et al*., 1999).

The use of IgY can also significantly reduce cross-reactivity and interference problems in immunological assays. Cross-reactivity in a multi-antibody assay may occur between IgG from different mammalian species. Because IgY is so different than IgG, no cross-reactivity should occur, and background will be reduced (Carlander *et al*., 1999). The use of IgY in immunological assays of serum samples can eliminate the interference and false positives normally experienced when using IgG. Newly obtained human serum samples often contain an active complement system, which would be activated by mammalian antibodies. IgY, on the other hand, does not activate the human complement system, and therefore its use can eliminate the interference that would be caused by IgG (Larsson *et al*., 1992a). Human serum samples may also contain rheumatoid factor (RF) and human anti-mouse IgG antibodies (HAMA), which are well-known causes of false positive reactions in immunological

assays (Carlander *et al*., 1999). RF is an autoantibody that reacts with the Fc portion of IgG, and HAMA can be found naturally in human serum, or in individuals treated with mouse antibodies for therapeutic purposes, and will bind to any mouse antibodies being used in an immunoassay. Both RF and HAMA may interfere by mimicking antigen activity or forming immune complexes with other antibodies being used in the assay (Carlander *et al*., 1999). IgY does not react with RF (Larsson and Sjöquist, 1988; Larsson *et al*, 1991) or HAMA (Larsson and Mellstedt, 1992), and their use has been suggested in place of IgG for immunological assays dealing with human serum. The Fc portion of mammalian IgG may also interact with the Fc receptor, found on many types of blood cells and bacteria. IgY does not interact with Fc receptors, and can therefore be used to avoid interference due to Fc binding (Carlander *et al*., 1999). Finally, as IgY does not bind protein A or G, it can be used to detect these without interference, as would otherwise be encountered when using IgG (Larsson *et al*., 1992b), and its use has been described for the detection and quantification of protein A in mouse monoclonal antibody preparations which have been purified using protein A (Godfrey *et al*., 1992).

Chicken antibodies have been used in many recent diagnostic applications, including: diagnosis of gastric cancer (Noack *et al*., 1999), detection of breast and ovarian cancer markers (Grebenschikov *et al*., 1997; Lemamy *et al*., 1999; Al-Haddad *et al*., 1999), detection of African horsesickness virus (Du Plessis *et al*., 1999), determination of hepatocyte growth factor (HGF) in serum and urine (Ohnishi *et al*., 2000), *Campylobacter* fetus diagnosis (Cipolla *et al*., 2001), detection of transforming growth factor (TGF) in biological fluids (Danielpour and Roberts, 1995), determination of reactants of inflammation (which would otherwise interfere with mammalian antibodies) (Rieger *et al*., 1996), detection of *Bordetella bronchiseptica* (Hlinak *et al*., 1996), and for the detection of human serum antigens using surface plasmon resonance (SPR) (Vikinge *et al*., 1998). Its use has recently been suggested for the detection of Human Papillomavirus for the early detection of cervical cancer (Di Lonardo *et al*., 2001), for the detection of the protein YRL-40 as a marker for disease models of arthritis, cancer, atherosclerosis, and liver fibrosis (De Ceuninck *et al*., 2001), and for use for hemoclassification (Gutierrez Calzada *et al*., 2001).

4.3 Immunoglobulin Y in immunoaffinity chromatography

Immunoaffinity chromatography involves the isolation and purification of target molecules, using immobilized antibodies directed against the target molecule. Due to the highly specific nature of the antibody-antigen interaction, immunoaffinity chromatography allows for the purification of specific molecules from complex starting materials. The widespread use of this process on a large scale, however, has been limited by the high cost of the technique, and parameters relating to the production of antibody and the efficiency of immobilization (Li-Chan, 2000). As chickens may be used to produce large quantities of highly specific antibodies, IgY would be an ideal replacement for other polyclonal or monoclonal antibodies currently used in immunoaffinity chromatography. Immobilized yolk antibodies have been used for the isolation of value-added proteins from dairy products, including the purification of lactoferrin (Li-Chan *et al*., 1998), and the isolation and separation of IgG subclasses from colostrum, milk, and cheese whey (Akita and Li-Chan, 1998). Although IgY is more sensitive to low pH than IgG, Akita and Li-Chan (1998) reported that, using standard affinity chromatography conditions (*i.e*. elution at low pH), an IgY immunoaffinity column was stable, and could be reused over 50 times without significant decreases in binding capacity. Alternative eluents have been examined, including highly alkaline conditions (Kuronen *et al*., 1997) and high concentrations of guanidine hydrochloride (Otani *et al*., 1991). Kummer and Li-Chan (1998) examined several eluents and elution conditions for IgY immunoaffinity chromatography. Different buffers (pH 2.3–7.5), various salts (NaCl, $(NH_4)_2SO_4$, and $MgCl_2$), ethylene glycol and glycerol, were compared to the traditionally used elution buffer (containing glycine, and at pH 2.8), and a commercially available eluent, Actisep. They found that the low pH buffers, Actisep, and $MgCl_2$, all dissociated IgY from immobilized IgG, however, the dissociation was to a lesser extent when dissociating IgG from immobilized IgY. As well, some denaturation was observed with $MgCl_2$ and Actisep, however denaturation of the IgY following exposure to low pH was not evident. To extend the use of IgY immunoaffinity columns, Kim *et al*. (1999) also examined the reusability of avidin-biotinylated IgY columns, in which biotinylated IgY is held by strong non-covalent interaction on columns containing immobilized avidin.

They found that when the antibody binding activity had been reduced by prolonged use, the column could be regenerated by dissociating the avidin-biotinylated IgY complex using guanidine hydrochloride and low pH, and applying new biotinylated IgY, thereby restoring the binding activity of the column.

A number of other applications using IgY immunoaffinity columns have been described for the purification of biological molecules from human serum, including the purification of tetrachlordibenzo-p-dioxin (Shelver *et al*., 1998), prekallikrein (Burger *et al*., 1986), and human alpha-2 antiplasmin (Lee *et al*., 1997).

4.4 Others

It has been estimated that 1.7 million people are bitten or stung by venomous snakes, scorpions, jelly fish, or spiders each year, resulting in 40 000−50 000 fatalities. The most widely used treatment of envenomation is the use of specific anti-venoms to neutralize the toxic and potentially lethal effects of the venom. Chicken anti-venom IgY has been produced, and was found to have a higher bioactivity than anti-venoms raised in horses (Thalley and Carroll, 1990; Almeida *et al*., 1998). IgY also has a lower likelihood of producing significant clinical side effects, such as serum sickness and anaphylactic shock, which can occur upon administration of mammalian serum proteins (Thalley and Carroll, 1990; Larsson *et al*., 1993).

Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases, which are an increasing burden to hospitals and society in terms of the cost of medication and treatment, and time lost due to illness (Hay and Hay, 1992). Standard medical care for these diseases includes anti-inflammatory drugs, immunosuppressants, and antibiotics, but their use is limited by side effects, immunosuppression, and incomplete efficacy. Immunotherapy using monoclonal mouse antibodies directed against tumor necrosis factor (TNF) has been approved for use, however it can be costly and adverse side effects have been reported in patients receiving systemic anti-TNF therapy (Sandborn and Hanauer, 1999). Recently Worledge *et al*. (2000) reported that anti-TNF antibodies produced in chickens were capable of effectively treating acute and chronic phases of colitis in rats, and were also found to neutralize human TNF *in vitro*, indicating its possible use for the treatment of inflammatory bowel disease in humans in the future.

As well, the production of IgY against barley yellow dwarf virus (Hu *et al*., 1985), influenza virus (Cuceanu *et al*., 1991), canine distemper virus (Schmidt *et al*., 1989), rabies virus (Sun *et al*., 2001), and mycotoxin (Kierek-Jaszczuk *et al*., 1997), as well as biological molecules such as antiplasmin (Lee *et al*., 1997) and actin (Schrader *et al*., 1994) have been reported.

5. CONCLUSIONS AND FUTURE PROSPECTS

It has long been known that chickens, like mammals, are capable of producing antigen-specific IgY, which functions similar to IgG, in response to an antigenic stimulus. It was not until recently, however, that the particular immunological properties of IgY were recognized, and IgY began replacing mammalian antibodies in such applications as immunodiagnostic assays and affinity purification techniques. However, that receiving the most attention has been the application of IgY as an immunotherapeutic agent. Prompted by the need to treat microorganisms which do not respond to traditional antibiotic therapy, IgY has been produced and tested against a number of bacterial and viral antigens. Treatment with IgY has been shown to provide a safer, more efficient, and less expensive method than antibiotics for managing disease-causing organisms. Yolk antibodies do not activate the mammalian complement system or interact with mammalian Fc receptors that could mediate inflammatory response in the gastrointestinal tract (Carlander *et al*., 2000). And although low levels of IgY were found in the circulation of piglets treated orally with IgY (Yokoyama *et al*., 1992b), no absorption of intact antibodies, via the gastrointestinal tract, has been shown in humans (Blum *et al*., 1981; Losonsky *et al*., 1985; Eibl *et al*., 1988) indicating that no systemic effects would be expected following the oral administration of IgY to humans (Carlander *et al*., 2000). However, few clinical trials involving the oral administration of specific IgY in humans have been carried out to date.

As these immunotherapeutic applications often require the continuous or frequent administration of antibodies, large quantities are required. IgY is therefore the ideal choice for the production of large quantities of conveniently purified antibodies. The use of IgY is also cost-effective, with IgY costing

less than $10 per gram compared to IgG which can cost upwards of $20 000 per gram (Sim *et al*., 2000). This technology will allow for new potential applications of IgY in medicine, public health, veterinary medicine, and food safety (Sim *et al*., 2000). The production of antigen-specific antibodies in egg yolk also has significant implications for nutraceutical and functional food development. However, to be effective for such applications, methods of IgY encapsulation will need to be further examined, as there is some controversy regarding the survival of IgY through the gastrointestinal tract and its implications for human immunotherapy. This would open the door for significant advances in IgY technology, such that the use of specific IgY could effect the widespread prevention and treatment of enteric diseases such as those due to *E. coli*, *Salmonella*, and rotavirus.

Research continues to be carried out on potential methods of production and application of egg yolk antibodies. Romito *et al*. (2001) suggested the immunization of chickens with naked DNA, to elicit antigen-specific IgY. Using DNA rather than a protein antigen could eliminate the protein expression and purification steps, and would allow the production of antibodies against pathogenic or toxic antigens. Not only are chickens useful for the production of specific IgY, but Mohammed *et al*. (1998) demonstrated the deposition of recombinant human antibodies into the egg yolk of transgenic chickens, suggesting an extension of the production of specific IgY in eggs. The production of monoclonal IgY has also been examined, generated through the fusion of spleen cells from immunized chickens with chicken B cells, to produce a monoclonal IgY-secreting hybridoma (Nishinaka *et al*., 1991; Asaoka *et al*., 1992; Lillehoj and Sasai, 1994; Nishinaka *et al*., 1996; Matsushita *et al*., 1998; Matsuda *et al*., 1999), capable of a consistent supply of antibody with a single and known specificity and homogeneous structure (Janeway and Travers, 1996). Lillehoj and Sasai (1994) and Kim *et al*. (2001) produced monoclonal IgY against *Eimeria* spp., an intracellular parasite responsible for avian coccidiosis, in order to study the avian immune response to this parasite, and to aid in vaccine development, as it was thought that monoclonal antibodies from mice would not adequately reflect the avian immune response, due to the differences in antibody repertoire. Due to the numerous advantages of IgY mentioned previously, monoclonal IgY would be ideal for use in diagnostic applications, where monoclonal mouse antibodies have traditionally been used. Several researchers have described the production of single chain fragment variable region (scFv) monoclonal IgY via recombinant DNA technology (Yamanaka *et al*., 1996; Nakamura *et al*., 2000; Kim *et al*., 2001), in order to improve upon the low levels of antibodies produced in the chicken hybridoma systems (Nakamura *et al*., 2000). Using RNA extracted from chicken hybridoma cells, Kim *et al*. (2001) were able to express recombinant monoclonal IgY in *E. coli*. and reported the production of 5–6 mg of IgY per litre of culture, suggesting that the production of monoclonal IgY on a large scale may be possible.

Since it is possible, using chickens, to produce antibodies against a vast array of antigens and epitopes, likely more than is possible in mammals due to the phylogenetic differences between the two, antibodies against any number of bacterial, viral, or biological antigens is possible, suggesting the significant potential of avian antibodies for further use in immunodiagnostics and identification of disease markers, immunotherapy and the treatment and prevention of disease, as well as affinity purification methods.

Despite the evidence for potential immunotherapeutic applications of IgY, and the many advantages it provides with respect to IgG for immunodiagnostics and immunoaffinity purification, mammalian antibodies continue to predominately be used, perhaps influenced by a lack of knowledge of the many benefits of IgY technology, unfamiliarity with chicken husbandry and the techniques involved in producing IgY, or simply due to convention. Tini *et al*. (2002) noted that considering the many benefits of IgY technology and its universal application in both research and medicine, it is expected that IgY will play an increasing role in research, diagnostics, and immunotherapy in the future.

REFERENCES

Akita, E.M. and Li-Chan, E.C.Y. (1998) Isolation of bovine immunoglobulin G subclasses from milk, colostrums, and whey using immobilized egg yolk antibodies. *J. Dairy Sci*., **81**, 54.

Akita, E.M. and Nakai, S. (1992) Immunoglobulins from egg yolk: Isolation and purification. *J. Food Sci*., **57**, 629.

Akita, E.M. and Nakai, S. (1993a) Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with an enterotoxigenic *E. coli* strain. *J. Immunol. Methods*, **160**, 207.

Akita, E.M. and Nakai, S. (1993b) Production and purification of Fab' fragments from chicken egg yolk immunoglobulin Y (IgY). *J. Immunol. Methods*, **162**, 155.

Al-Haddad, S., Zhang, Z., Leygue, E., Snell, L., Huang, A., Niu, Y., Hiller-Hitchcock, T., Hole, K. and Murphy, L.C. (1999) Psoriasin (S100A7) expression and invasive breast cancer. *Am. J. Pathol*., **155**, 2057.

Almeida, C.M., Kanashiro, M.M., Rangel Filho, F.B., Mata, M.F. and Kipnis, T.L. (1998) Development of snake antivenom antibodies in chickens and the purification from yolk. *Vet. Rec*., **143**, 579.

Asaoka, H., Nishinaka, S., Wakamiya, N., Matsuda, H. and Murata, M. (1992) Two chicken monoclonal antibodies specific for heterophil Hanganutziu-Deicher antigens. *Immunol. Lett*., **32**, 91.

Bade, H. and Stegemann, H. (1984) Rapid method of extraction of antibodies from hen egg yolk. *J. Immunol. Methods*, **71**, 421.

Barrett, J.T. (1983) Textbook of *Immunology, An Introduction to Immunochemistry and Immunobiology, Fourth Edition*. The C.V. Mosby Company, St. Louis.

Bell, C. and Kriakides, A. (1998) *Salmonella: A practical approach to the organism and its control in foods*. Blackie, A & P, New York.

Benjamini, E., Sunshine, G. and Leskowitz, S. (1996) *Immunology, A Short Course, Third Edition*. Wiley-Liss, Inc., New York.

Bizhanov, G. and Vyshniauskis, G. (2000) A comparison of three methods for extracting IgY from the egg yolk of hens immunized with Sendai virus. *Vet. Res. Commun*., **24**, 103.

Blum, P.M., Phelps, D.L., Ank, B.J., Krantman, H.J. and Stiehm, E.R. (1981) Survival of oral human immune serum globulin in the gastrointestinal tract of low birth weight infants. *Pediatr. Res*., **15**, 1256.

Both, G.W., Bellamy, A.R., and Mitchell, D.B. (1994) Rotavirus protein structure and function, In *Rotaviruses*, ed. Ramig, R.F. Springer-Verlag, New York, p. 67.

Burger, D., Schleuning, W.D. and Schapira, M. (1986) Human plasma prekallikrein. Immunoaffinity purification and activation to alpha- and beta-kallikrein. *J. Biol. Chem*., **261**, 324.

Burley, R.W. and Cook, W.H. (1961) Isolation and composition of avian egg yolk granules and their constituents α- and *β*-lipovitellines. *Can. J. Biochem. Physiol*., **39**, 1295.

Burley, R.W. and Vadehra, D.V. (1989) An outline of the physiology of avian eggs, In *The Avian Egg: Chemistry and Biology*, Wiley International, New York, p. 17.

Carlander, D., Stalberg, J. and Larsson, A. (1999) Chicken antibodies: a clinical chemistry perspective. *Ups. J. Med. Sci*., **104**, 179.

Carlander, D., Kollberg, H., Wejaker P.-E. and Larsson, A. (2000) Peroral immunotherapy with yolk antibodies for the prevention and treatment of enteric infections. *Immunol. Res*., **21**, 1.

Casadevall, A. and Scharff, M.D. (1995) Return to the past: the case for antibody-based therapies in infectious diseases. *Clin. Infect. Dis*., **21**, 150.

Chang, H.M., Ou-Yang, R.F., Chen, Y.T. and Chen, C.C. (1999) Productivity and some properties of immunoglobulin specific against *Streptococcus mutans* serotype c in chicken egg yolk (IgY). *J. Agric. Food Chem*., **47**, 61.

Chang, H.M., Lu, T.C., Chen, C.C., Tu, Y.Y. and Hwang, J.Y. (2000) Isolation of immunoglobulin from egg yolk by anionic polysaccharides. *J. Agric. Food Chem*., **48**, 995.

Cipolla, A., Cordeviola, J., Terzolo, H., Combessies, G., Bardon, J., Ramon, N., Martinez, A., Medina, D., Morsella, C. and Malena, R. (2001) Campylobacter fetus diagnosis: direct immunofluorescence comparing chicken IgY and rabbit IgG conjugates. *ALTEX*, **18**, 165.

Coleman, M. (2000) Using egg antibodies to treat diseases, In *Egg Nutrition and Biotechnology*, eds Sim, J.S., Nakai, S. and Guenter, W. CABI Publishing, New York, p. 351.

Cotterill, O.J. and McBee, L.E. (1995) Egg breaking, In *Egg Science and Technology*, Fourth Edition, eds Stadelman, W.J. and Cotterill, O.J. The Haworth Press, Inc., New York, p. 231.

Crabb, J.H. (1998) Antibody-based immunotherapy of cryptosporidiosis. *Adv. Parasitol*., **40**, 121.

Cuceanu, N., Constantinescu, C. and Ionita, E. (1991) Isolation and characterization of egg yolk antibodies IgY from immunized with different influenza virus strains. *Roum. Arch. Microbiol. Immunol*., **50**, 215.

Danielpour, D. and Roberts, A.B. (1995) Specific and sensitive quantification of transforming growth factor by sandwich enzyme-linked immunosorbent assay. *J. Immunol. Methods*, **180**, 265.

De Ceuninck, F., Pastoureau, P., Agnellet, S., Bonnet, J. and Vanhoutte, P.M. (2001) Development of an enzyme-linked immunoassay for the quantification of YKL-40 (cartilage gp-39) in guinea pig serum using hen egg yolk antibodies. *J. Immunol. Methods*, **252**, 153.

Deignan, T., Kelly, J., Alwan, A. and O'Farrelly, C. (2000) Comparative analysis of methods of purification of egg yolk immunoglobulin. *Food Agric. Immunol*., **12**, 77.

Desselberger, U. and McCrae, M.A. (1994) The Rotavirus Genome, In *Rotaviruses*, ed. Ramig, R.F.Springer-Verlag, New York, p. 31.

Di Lonardo, A., Luisa Marcante, M., Poggiali, F., Hamsøikovà, E., and Venuti, A. (2001) Egg yolk antibodies against the E7 oncogenic protein of human papillomavirus type 16. *Arch. Virol*., **146**, 117.

Du Plessis, D.H., Van Wyngaardt, W., Romito, M., Du Plessis, M. and Maree, S. (1999) The use of chicken IgY in a double sandwich ELISA for detecting African horsesickness virus. *Onderstepoort J. Vet. Res*., **66**, 25.

Ebina, T. (1996) Prophylaxis of rotavirus gastroenteritis using immunoglobulin. *Arch. Virol. Suppl*., **12**, 217.

Eibl, M.M., Wolf, H.M., Furnkranz, H. and Rosenkranz, A. (1988) Prevention of necrotizing enterocolitis in low birth-weight infants by IgA-IgG feeding. *N. Engl. J. Med*., **319**, 1.

Epple, A. and Steson, M. (1980) *Avian endocrinology*. Academic Press, New York.

Erhard, M.E., Schmidt, P., Hofmann, A., Stangassinger, M. and Losch, U. (1996) Production and purification of mouse IgG subclass specific chicken egg yolk antibodies using a new indirect affinity chromatography method with protein G Sepharose. *ALTEX*, **13**, 66.

Eterradossi, N., Toquin, D., Abbassi, H., Rivallan, G., Cotte, J.P. and Guittet, M. (1997) Passive protection of specific pathogen free chicks against infectious bursal disease by in-ovo injection of semi-purified egg-yolk antiviral immunoglobulins. *J. Vet. Med*., **B44**, 371.

Fassina, G., Verdoliva, A., Palombo, G., Ruvo, M. and Cassani, G. (1998) Immunoglobulin specificity of TG19318: a novel synthetic ligand for antibody affinity purification. *J. Mol. Recognit*., **11**, 128.

Fichtali, J., Charter, E.A., Lo, K.V. and Nakai, S. (1992) Separation of egg yolk immunoglobulins using an automated liquid chromatography system. *Biotech. Bioeng*., **40**, 1388.

Fichtali, J., Charter, E.A., Lo, K.V. and Nakai, S. (1993) Purification of antibodies from industrially separated egg yolk. *J. Food Sci*., **58**, 1282.

Galdiero, F., Tufano, M.A., Galdiero, M., Masiello, S. and Rosa, M.D. (1990) Inflammatory effects of *Salmonella typhimurium* porins. *Infect. Immun*., **58**, 3183.

Gardner, P.S. and Kaya, S. (1982) Egg globulin in rapid virus diagnosis. *J. Virol. Methods*, **4**, 257.

Gassmann, M., Thommes, P., Weiser, T. and Hubscher, U. (1990) Efficient production of chicken egg yolk antibodies against a conserved mammalian protein. *FASEB J*. **4**, 2528.

Godfrey, M.A., Kwasowski, P., Clift, R. and Marks, V. (1992) A sensitive enzyme-linked immunosorbent assay (ELISA) for the detection of staphylococcal protein A (SpA) present as a trace contaminant of murine immunoglobulins purified on immobilized protein A. *J. Immunol. Methods*, **149**, 21.

Gottstein, B. and Hemmeler, E. (1985) Egg yolk immunoglobulin Y as an alternative antibody in the serology of echinococcosis. *Z. Parasitenkd*, **71**, 273.

Grebenschikov, N., Geurts-Moespot, A., De Witte, H., Heuvel, J., Leake, R., Sweep, F. and Benraad, T. (1997) A sensitive and robust assay for urokinase and tissue-type plasminogen activators (uPA and tPA) and their inhibitor type I (PAI-1) in breast tumor cytosols. *Int. J. Biol. Markers*, **12**, 6.

Greene, C.R. and Holt, P.S. (1997) An improved chromatographic method for the separation of egg yolk IgG into subpopulations utilizing immobilized metal ion (Fe^{3+}) affinity chromatography. *J. Immunol. Methods*, **209**, 155.

Gutierrez, M.A., Miyazaki, T., Hatta, H. and Kim, M. (1993) Protective properties of egg yolk IgY containing anti-*Edwardsiella tarda* antibody against paracolo disease in the Japanese eel, Anguilla japanica Temminck & Schlegel. *J. Fish Dis*., **16**, 113.

Gutierrez Calzado, E., Garcia Garrido, R.M. and Schade, R. (2001) Human haemoclassification by use of specific yolk antibodies obtained after immunization of chickens against human blood group antigens. *Altern. Lab. Anim*., **29**, 717.

Hamada, S. and Slade, H.D. (1980) Biology, immunology, and cariogenicity of Streptococcus mutans. *Microbiol. Rev*., **44**, 331.

Hamada, S., Horikoshi, T., Minami, T., Kawabata, S., Hiraoka, J., Fujiwara, T. and Ooshima, T. (1991) 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*., **59**, 4161.

Hammarstrom, L. (1999) Passive immunity against rotavirus in infants. *Acta Paediatr. Suppl*., **430**, 127.

Hampson, D.J. (1994) Postweaning *Escherichia coli* diarrhoea in pigs, In *Escherichia coli in Domestic Animals and Humans*, ed. Gyles, C.L. CAB International, Oxon, UK, p. 171.

Hanley, W.C., Artwohl, J.E. and Bennett, B.T. (1995) Review of polyclonal antibody production procedures in mammals and poultry. *ILAR J*., **37**, 93.

Hansen, P., Scoble, J.A., Hanson, B. and Hoogenraad, N.J. (1998) Isolation and purification of immunoglobulins from chicken eggs using thiophilic interaction chromatography. *J. Immunol. Methods*, **215**, 1.

Hassl, A. and Aspock, H. (1988) Purification of egg yolk immunoglobulins. A two-step procedure using hydrophobic interaction chromatography and gel filtration. *J. Immunol. Methods*, **110**, 225.

Hatta, H., Sim, J.S. and Nakai, S. (1988) Separation of phospholipids from egg yolk and recovery of water-soluble proteins. *J. Food Sci*., **53**, 425.

Hatta, H., Kim, M. and Yamamoto, T. (1990) A novel isolation method for hen egg yolk antibody ''IgY''. *Agric. Biol. Chem*., **54**, 2531.

Hatta, H., Tsuda, K., Akachi, S., Kim, M., Yamamoto, T. and Ebina, T. (1993) Oral passive immunization effect of anti-human rotavirus IgY and its behavior against proteolytic enzymes. *Biosci. Biotech. Biochem*., **57**, 1077.

Hatta, H., Mabe, K., Kim, M., Yamamoto, T., Gutierrez, M.A. and Miyazaki, T. (1994) Prevention of fish disease using egg yolk antibody, In *Egg Uses and Processing Technologies, New Developments*, eds Sim, J.S. and Nakai, S. CAB International, Oxon, UK, p. 241.

Hatta, H., Tsuda, K., Ozeki, M., Kim, M., Yamamoto, T., Otake, S., Hirosawa, m., Katz, J., Childers, N.K. and Michalek, S.M. (1997a) 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.

Hatta, H., Ozeki, M. and Tsuda, K. (1997b) Egg yolk antibody IgG and its application, In *Hen Eggs; Their basic and applied science*, eds Yamamoto, T., Juneja, L.R., Hatta, H., and Kim, M. CRC press, New York, p. 151.

Hay, J.W. and Hay, A.R. (1992) Inflammatory bowel disease: costs-of-illness. *J. Clin. Gastroenterol*., **14**, 309.

Hersh, R.T. and Benedict, A.A. (1966) Aggregation of chicken gamma-G immunoglobulin in 1.5 M sodium chloride solution. *Biochim. Biophys. Acta*, **115**, 242.

Hilpert, H., Gerber, H., Pahud, J.J., Ballabriga, A., Arcalis, L., Farriaux, F., de Leyer, E. and Nussle, D. (1977) Bovine milk immunoglobulins (Ig). Their possible utilization in industrially prepared infant's milk formula, In *Food and Immunology*, eds Hambraeusm, L., Hanson, L. and McFarlene, H. Almquist and Wiksell International, Stockholm, Sweden, p. 182.

Hlinak, A., Kruger, M., Bartels, T., Sasse, M., Claros, M. and Schade, R. (1996) Studies on diagnostic applications of egg yolk antibodies against Bordetella bronchiseptica. *ALTEX*, **13**, 70.

Hochwald, C. and Kivela, L. (1999) Rotavirus vaccine, live, oral, tetravalent (RotaShield). *Pediatr. Nurs*., **25**, 203.

Horikoshi, T., Hiraoka, J., Saito, M. and Hamada, S. (1993) IgG antibody from hen egg yolk: Purification by ethanol fractionation. *J. Food Sci*., **58**, 739.

Hu, J.S., Rochow, W.F. and Dietert, R.R. (1985) Production and use of antibodies from hen eggs for the SGV isolate of barley yellow dwarf virus. *Phytopathology*, **75**, 914.

Ikemori, Y., Kuroki, M., Peralta, R.C., Yokoyama, H. and Kodama, Y. (1992) 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*., **53**, 2005.

Ikemori, Y., Peralta, R.C., Kuroki, M., Yokoyama, H. and Kodama, Y. (1993) Avidity of chicken yolk antibodies to enterotoxigenic *Escherichia coli* fimbriae. *Poultry Sci*., **72**, 2361.

Ikemori, Y., Ohta, M., Umeda, K., Icatlo, F.C. Jr., Kuroki, M., Yokoyama, H., and Kodama, Y. (1997) Passive protection of neonatal calves against bovine coronavirus-induced diarrhea by administration of egg yolk or colostrum antibody powder. *Vet. Microbiol*., **58**, 105.

Imberechts, H., Deprez, P., Van Driessche, E. and Pohl, P. (1997) Chicken egg yolk antibodies against F18ab fimbriae of *Escherichia coli* inhibit shedding of F18 positive *E. coli* by experimentally infected pigs. *Vet. Microbiol*., **54**, 329.

Isibasi, A., Ortiz, V., Vargas, M., Paniagua, J., Gonzales, C., Moreno, J. and Kumate, J. (1988) Protection against Salmonella typhi infection in mice after immunization with outer membrane proteins isolated from *Salmonella typhi* 9, 12, d, Vi. *Infect. Immun*., **56**, 2953.

Janeway, C.A. and Travers, P. (1996) *Immunobiology, The Immune System in Health and Disease, Second Edition*. Garland Publishing Inc., New York.

Janson, A.K., Smith, C.I. and Hammarstrom, L. (1995) Biological properties of yolk immunoglobulins. *Adv. Exp. Med. Biol*., **371**, 685.

Jensenius, J.C., Anderson, I., Hau, J., Crone, M. and Kock, C. (1981) Eggs: conveniently packaged antibodies. Method for purification of yolk IgG. *J. Immunol. Methods*, **46**, 63.

Jensenius, J.C. and Koch, C. (1993) On the purification of IgG from egg yolk. *J. Immunol. Methods*, **164**, 141.

Jin, L.Z., Samuel, K., Baidoo, K., Marquardt, R.R. and Frohlich, A.A. (1998) *In vitro* inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 to piglet intestinal mucus by egg yolk antibodies. *FEMS Immunol. Medical Microbiology*, **21**, 313.

Juneja, L.R. (1997) Egg yolk lipids, In *Hen Eggs; Their basic and applied science*, eds Yamamoto, T., Juneja, L.R., Hatta, H. and Kim, M. CRC press, New York, p. 73.

Kapikian, A.Z, and Chanock, R.M. (1996) Rotaviruses, In *Fields Virology*, Third Edition, eds Fields, B.N., Knipe, D.N., Howley, P.M., Chanock, R.M., Melnick, J.L., Monath, T.P., Rolzman, B. and Strauss, S.E. Raven Press, New York, NY. p. 1657.

Kapil, S, Trent, A.M. and Goyal, S.M. (1990) Excretion and persistence of bovine coronavirus in neonatal calves. *Arch. Virol*., **115**, 127.

Kierek-Jaszczuk, D., Marquardt, R.R. and Abramson, D. (1997) Use of a heterologous solid-phase antigen in an indirect competitive antibody-capture enzyme–linked immunosorbent assay for T-2 mycotoxin. *J. Food Protec*., **60**, 321.

Kim, H. and Nakai, S. (1996) Immunoglobulin separation from egg yolk: a serial filtration system. *J. Food Sci*., **61**, 510.

Kim, H. and Nakai, S. (1998) Simple separation of immunoglobulin from egg yolk by ultrafiltration. *J. Food Sci*., **63**, 485.

Kim, H., Durance, T.D. and Li-Chan, E.C.Y. (1999) Reusability of avidin-biotinylated immunoglobulin Y columns in immunoaffinity chromatography. *Anal. Biochem*., **268**, 383.

Kim, J.-K., Min, W., Lillehoj, H.S., Kim, S., Sohn, E.J., Song, K.D. and Han, J.Y. (2001) Generation and characterization of recombinant scFv antibodies detecting *Eimeria acervulina* surface antigens. *Hybridoma*, **20**, 175.

Kovacs-Nolan, J., Sasaki, E., Yoo, D. and Mine, Y. (2001) Cloning and expression of human rotavirus spike protein, VP8*, in *Escherichia coli*. *Biochem. Biophys. Res. Commun*., **282**, 1183.

Kronvall, G., Seal, U.S., Svensson, S. and Williams, R.C. Jr. (1974) Phylogenetic aspect of *Staphylococcal* protein A-reactive serum globulins in birds and mammals. *Acta. Path. Microbiol. Scand. Section B*, **82**, 12.

Kuroki, M., Ohta, Y., Ikemori, Y., Peralta, R.C., Yokoyama, H. and Kodama, Y. (1994) Passive protection against bovine rotavirus in calves by specific immunoglobulins from chicken egg yolk. *Arch. Virol*., **138**, 143.

Kummer, A. and Li-Chan, E.Y.C. (1998) Application of an ELISA-elution assay as a screening tool for dissociation of yolk antibody-antigen complexes. *J. Immunol. Methods*, **211**, 125.

Kuronen, I., Kokko, H., Mononen, I. And Parviainen, M. (1997) Hen egg yolk antibodies purified by antigen affinity under highly alkaline conditions provide new tools for diagnostics. Human intact parathyrin as a model antigen. *Eur. J. Clin. Chem. Clin. Biochem*., **35**, 435.

Kwan, L., Li-Chan, E., Helbig, N. and Nakai, S. (1991) Fractionation of water-soluble and -insoluble components from egg yolk with minimum use of organic solvents. *J. Food Sci*., **56**, 1537.

Kweon, C.-H, Kwon, B.-J., Woo, S.-R., Kim, J.-M., Woo, G.-H., Son, D.H., Hur, W. and Lee, Y.-S. (2000) Immunoprophylactic effect of chicken egg yolk immunoglobulin (IgY) against porcine epidemic diarrhea virus (PEDV) in piglets. *J. Vet. Med. Sci*., **62**, 961.

Larsson, A. and Sjöquist, J. (1988) Chicken antibodies: A tool to avoid false positive results by rheumatoid factor in latex fixation tests. *J. Immunol. Methods*, **108**, 205.

Larsson, A., Carlander, D. and Wilhelmsson, M. (1988) Antibody response in laying hens with small amounts of antigen. *Food Agr. Immunol*., **10**, 29.

Larsson, A. and Sjöquist, J. (1990) Chicken IgY: Utilizing the evolutionary difference. *Comp. Immun. Microbiol. Infect. Dis*., **13**, 199.

Larsson, A., Karlsson-Parra, A. and Sjöquist, J. (1991) Use of chicken antibodies in enzyme immunoassays to avoid interference by rheumatoid factors. *Clin. Chem*., **37**, 411.

Larsson, A., Wejaker, P.E., Forsberg, P.O. and Lindahl, T. (1992a) Chicken antibodies: a tool to avoid interference by complement activation in ELISA. *J. Immunol. Methods*, **156**, 79.

Larsson, A., Wejaker, P.E., and Sjöquist, J. (1992b) Chicken anti-protein A for the detection and capturing of protein A from *Staphylococcus aureus* in the presence or absence of mammalian IgG. *Hybridoma*, **11**, 239.

Larsson, A. and Mellstedt, H. (1992) Chicken antibodies: a tool to avoid interference by human anti-mouse antibodies in ELISA after *in vivo* treatment with murine monoclonal antibodies. *Hybridoma*, **11**, 33.

Larsson, A., Balow, R., Lindahl, T.L. and Forsberg, P. (1993) Chicken antibodies: taking advantage of evolution; a review. *Poult. Sci*., **72**, 1807.

Lee, J., Babiuk, L.A., Harland, R., Gibbons, E., Elazhary, Y. and Yoo, D. (1995) Immunological response to recombinant VP8* subunit protein of bovine rotavirus in pregnant cattle. *J. Gen. Virol*., **76**, 2477.

Lee, S.C., Lee, K.N., Schwartzott, D.G., Jackson, K.W., Tae, W.-C. and McKee, P.A. (1997) Purification of human alpha 2-antiplasmin with chicken IgY specific to its carboxy-terminal peptide. *Prep. Biochem. Biotechnol*., **27**, 227.

Lee, S.B., Mine, Y. and Stevenson, R.M.W. (2000) Effects of hen egg yolk immunoglobulin in passive protection of rainbow trout against *Yersinia ruckeri*. *J. Agric. Food Chem*., **48**, 110.

Lemamy, G.J., Roger, P., Mani, J.C., Robert, M., Rochefort, H. and Brouillet, J.P. (1999) High-affinity antibodies from hen's egg-yolks against human mannose-6- phosphate/insulin-like growth-factor-II receptor (M6P/IGFII-R): characterization and potential use in clinical cancer studies. *Int. J. Cancer*, **80**, 896.

Leslie, G.A. and Clem, L.W. (1969) Phylogeny of immunoglobulin structure and function. III Immunoglobulins of the chicken. *J. Exp. Med*., **130**, 1337.

Leslie, G.A. and Martin, L.N. (1973) Studies on the secretory immunologic system of fowl. 3. Serum and secretory IgA of the chicken. *J. Immunol*., **110**, 1.

Li, X., Nakano, T., Sunwoo, H.H., Paek, B.H., Chae, H.S. and Sim, J.S. (1997) Effects of egg and yolk weights on yolk antibody (IgY) production in laying chickens. *Poultry Sci*., **77**, 266.

Li-Chan, E.C.Y. (2000) Applications of egg immunoglobulins in immunoaffinity chromatography, In *Egg Nutrition and Biotechnology*, eds Sim, J.S., Nakai, S. and Guenter, W. CAB International, New York, p. 323.

Li-Chan, E.Y.C., Powrie, W.D. and Nakai, S. (1995) The chemistry of eggs and egg products, In *Egg Science and Technology*, Fourth Edition, eds Stadelman, W.J. and Cotterill, O.J. The Haworth Press, Inc., New York, p. 105.

Li-Chan, E.C.Y., Ler, S.S., Kummer, A. and Akita, E.M. (1998) Isolation of lactoferrin by immunoaffinity chromatography using yolk antibodies. *J. Food Biochem*., **22**, 179.

Lillehoj, H.S. and Sasai, K. (1994) Development and characterization of chicken-chicken B cell hybridomas secreting monoclonal antibodies that detect sporozoite and merozoite antigens of *Eimeria*. *Poultry Sci*., **73**, 1685.

Loeken, M.R. and Roth, T.F. (1983) Analysis of maternal IgG subpopulations which are transported into the chicken oocyte. *Immunology*, **49**, 21.

Losonsky, G.A., Johnson, J.P., Winkelstein, J.A. and Yolken, R.H. (1985) Oral administration of human serum immunoglobulin in immunodeficient patients with viral gastroenteritis. A pharmacokinetic and functional analysis. *J. Clin. Invest*., **76**, 2362.

Ludert, J.E., Krishnaney, A.A., Burns, J.W., Vo, P.T. and Greenberg, H.B. (1996) Cleavage of rotavirus VP4 *in vivo*. *J. Gen. Virol*., **77**, 391.

Mackow, E.R., Shaw, R.D., Matsui, S.M., Vo, P.T., Dang, M.-N. and Greenberg, H.B. (1988) The rhesus rotavirus gene encoding protein VP3: location of amino acids involved in homologous and heterologous rotavirus neutralization and identification of a putative fusion region. *Proc. Natl. Acad. Sci. U.S.A*, **85**, 645.

Marchalonis, J.J. (1977) *Immunity in Evolution*. Harvard University Press, Cambridge, Massachusetts.

Marquardt, R.R., Jin, L.Z., Kim, J.W., Fang, L., Frohlich, A.A. and Baidoo, S.K. (1999) Passive protective effect of egg yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early weaned piglets. *FEMS Immunol. Medical Microbiology*, **23**, 283.

Matsuda, H., Mitsuda, H., Nakamura, N., Furusawa, S., Mohri, S. and Kitamoto, T. (1999) A chicken monoclonal antibody with specificity for the N-terminal of human prion protein. *FEMS Immunol. Med. Microbiol*., **23**, 189.

Matsushita, K., Horiuchi, H., Furusawa, S., Horiuchi, M., Shinagawa, M. and Matsuda, H. (1998) Chicken monoclonal antibodies against synthetic bovine prion protein peptide. *J. Vet. Med. Sci*., **60**, 777.

Matsuura, F., Ohta, M., Murakami, K. and Matsuki, Y. (1993) Structures of asparagines linked oligosaccharides of immunoglobulins (IgY) isolated from egg-yolk of Japanese quail. *Glycoconj. J*., **10**, 202.

McBee, L.E. and Cotterill, O.J. (1979) Ion-exchange chromatography and electrophoresis of egg yolk proteins. *J. Food Sci*., **44**, 656.

McCannel, A.A. and Nakai, S. (1989) Isolation of egg yolk immunoglobulin-rich fractions using copper-loaded metal chelate interaction chromatography. *Can. Inst. Food Sci. Technol. J*., **22**, 487.

McCannel, A.A. and Nakai, S. (1990) Separation of egg yolk immunoglobulins into subpopulations using DEAE-ion exchange chromatography. *Can Inst. Food Sci. Technol. J*., **23**, 42.

McLaren, R.D., Prosser, C.G., Grieve, R.C.J. and Borissenko, M. (1994) The use of caprylic acid for the extraction of the immunoglobulin fraction from egg yolk of chickens immunised with bovine α-lactalbumin. *J. Immunol. Methods*, **177**, 175.

Mine, Y. (1997) Separation of *Salmonella enteritidis* from experimentally contaminated liquid eggs using a hen IgY immobilized immunomagnetic separation system. *J. Agric. Food Chem*., **45**, 3723.

Mohammed, S.M., Morrison, S., Wims, L., Ryan Trinh, K., Wildeman, A.G., Bonselaar, J. and Etches, R.J. (1998) Deposition of genetically engineered human antibodies into the egg yolk of hens. *Immunotechnology*, **4**, 115.

Moon, H.W., Whipp, S.C. and Skartvedt, S.M. (1976) Etiologic diagnosis of diarrheal diseases of calves: frequency and methods for detecting enterotoxic and K99 antigen produced by *Escherichia coli*. *Am. J. Vet. Res*., **37**, 1025.

Morris, J.A. and Sojka, W.J. (1985) *Escherichia coli* as a pathogen in animals, In *The Virulence of Escherichia coli*, ed. Sussman, M. Academic Press, Inc., London, p. 47.

Morrison, S.L., Mohammed, M.S., Wims, L.A., Trinh, R. and Etches, R. (2002) Sequences in antibody molecules important for receptor-mediated transport into the chicken egg yolk. *Mol. Immunol*., **38**, 616.

Nakai, S., Li-Chan, E. and Lo, K.V. (1994) Separation of immunoglobulin from egg yolk, In *Egg Uses and Processing Technologies. New Developments*, eds Sim, J.S. and Nakai, S. CAB International, Wallingford, UK, p. 94.

Nakamura, N., Aoki, Y., Horiuchi, H., Furusawa, S., Yamanaka, H.I., Kitamoto, T. and Matsuda, H. (2000) Construction of

recombinant monoclonal antibodies from a chicken hybridoma line secreting specific antibody. *Cytotechnology*, **32**, 191.

Nejmeddine, M., Trugnan, G., Sapin, C., Kohli, E., Svensson, L., Lopez, S. and Cohen, J. (2000) Rotavirus spike protein VP4 is present at the plasma membrane and is associated with microtubules in infected cells. *J. Virol*., **74**, 3313.

Nishinaka, S., Suzuki, T., Matsuda, H. and Murata, M. (1991) A new cell line for the production of chicken monoclonal antibody by hybridoma technology. *J. Immunol. Methods*, **139**, 217.

Nishinaka, S., Akiba, H., Nakamura, M., Suzuki, K., Suzuki, T., Tsubokura, K., Horiuchi, H., Furusawa, S. and Matsuda, H. (1996) Two chicken B cell lines resistant to ouabain for the production of chicken monoclonal antibodies. *J. Vet. Med. Sci*., **58**, 1053.

Noack, F., Helmecke, D., Rosenberg, R., Thorban, S., Nekarda, H., Fink, U., Lewald, J., Stich, M., Schutze, K., Harbeck, N., Magdolen, V., Graeff, H. and Schmitt, M. (1999) CD87-positive tumor cells in bone marrow aspirates identified by confocal laser scanning fluorescence microscopy. *Int. J. Oncol*., **15**, 617.

O'Farrelly, C., Branton, D. and Wanke, C.A. (1992) Oral ingestion of egg yolk immunoglobulin from hens immunized with an enterotoxigenic *Escherichia coli* strain prevents diarrhea in rabbits challenged with the same strain. *Infect. Immun*., **60**, 2593.

Ohnishi, T., Kakimoto, K., Hashida, S., Fujii, M., Hirono, S., Nishiyama, K., Amita, Y., Ishikawa, E., Tsubouchi, H. and Daikuhara, Y. (2000) Development of highly sensitive enzyme-linked immunosorbent assays for hepatocyte growth factor/scatter factor (HGF/SF): determination of HGF/SF in serum and urine from normal human subjects. *J. Immunol. Methods*, **244**, 163.

Ohta, M., Hamako, J., Yamamoto, S., Hatta, H., Kim, M., Yamamoto, T., Oka, S., Mizuochi, T. and Matsuura, F. (1991) Structure of asparagine-linked oligosaccharides from hen egg yolk antibody (IgY). Occurrence of unusual glucosylated oligo-mammnose type oligosaccharides in a mature glycoprotein. *Glycoconj. J*., **8**, 400.

Olovsson, M. and Larsson, A. (1993) Biotin labelling of chicken antibodies and their subsequent use in ELISA and immunohistochemistry. *Comp. Immunol. Microbiol. Infect. Dis*., **16**, 145.

Otake, S., Nishihara, Y., Makimura, M., Hatta, H., Kim, M., Yamamoto, T. and Hirasawa, M. (1991) Protection of rats against dental caries by passive immunization with hen egg yolk antibody (IgY). *J. Dent. Res*., **70**, 162.

Otani, H., Matsumoto, K., Saeki, A. and Hosono, A. (1991) Comparative studies on properties of hen egg yolk IgY and rabbit serum IgG antibodies. *Lebensm. Wiss. Technol*., **24**, 152.

Parry, S.H. and Rooke, D.M. (1985) Adhesins and colonization factors of *Escherichia coli*, In *The Virulence of Escherichia coli*, ed. Sussman, M. Academic Press, Inc., London, p. 79.

Parvari, R., Avivi, A., Lentner, F., Ziv, E., Tel-Or, S., Burstein, Y. and Schechter, I. (1988) Chicken immunoglobulin G-heavy chains: limited VH gene repertoire, combinatorial diversification by D gene segments and evolution of the heavy chain locus. *EMBO J*., **7**, 739.

Peralta, R.C., Yokoyama, H., Ikemori, Y., Kuroki, M. and Kodama, Y. (1994) Passive immunization against experimental salmonellosis in mice by orally administered hen egg yolk antibodies specific for 14-kDa fimbriae of *Salmonella enteritidis*. *J. Med. Microbiol*., **41**, 29.

Pilz, I., Schwarz, E. and Palm, W. (1977) Small-angle X-ray studies of the human immunoglobulin molecule. *Eur. J. Biochem*., **75**, 195.

Pink, J.R.L., Vainio, O., and Rijnbeek, A.-M. (1985) Clones of B lymphocytes in individual follicles of the bursa of Fabricius. *Eur. J. Immunol*., **15**, 83.

Polson, A. (1990) Isolation of IgY from the yolks of eggs by a chloroform polyethylene glycol procedure. *Immunol. Invest*., **19**, 253.

Polson, A., Coetzer, T., Kruger, J., vov Maltzahn, E. and van der Merwe, K.J. (1985) Improvements in the isolation of IgY from the yolks of eggs laid by immunized hens. *Immunol. Invest*., **14**, 323.

Polson, A., von Wechmar, M.B. and Fazakerley, G. (1980a) Antibodies to proteins from yolk of immunized hens. *Immunol. Commun*., **9**, 495.

Polson, A., von Wechmar, M.B. and van Regenmortel, M.H. (1980b) Isolation of viral IgY antibodies from yolks of immunized hens. *Immunol. Commun*., **9**, 475.

Prasad, B.V.V., Burns, J.W., Marietta, E., Estes, M.K. and Chiu, W. (1990) Localization of VP4 neutralization sites in rotavirus by three-dimensional cryo-electron microscopy. *Nature*, **343**, 476.

Reiger, A., Burger, W., Hiepe, F. and Schade, R. (1996) Determination of human serum CRP using a chicken egg yolk antibody. *ALTEX*, **13**, 57.

Reilly, R.M., Domingo, R. and Sandhu, J. (1997) Oral delivery of antibodies; Future pharmacokinetic trends. *Clin. Pharmacokinet*., **4**, 313.

Reynaud, C.-A., Dahan, A. and Weill, J.-C. (1983) Complete sequence of a chicken λ light chain immunoglobulin derived from the nucleotide sequence of its mRNA. *Proc. Natl. Acad. Sci. USA*, **80**, 4099.

Reynaud, C.-A., Dahan, A., Anquez, V. and Weill, J.-C. (1985) A single rearrangement event generates most of the chicken immunoglobulin light chain diversity. *Cell*, **40**, 283.

Reynaud, C.-A., Anquez, V., Grimal, H. and Weill, J.-C. (1987) A hyperconversion mechanism generates the chicken light chain preimmune repertoire. *Cell*, **48**, 379.

Reynaud, C.-A., Anquez, V. and Weill, J.-C. (1989) Somatic hyperconversion diversifies the single VH gene of the chicken with a high incidence in the D region. *Cell*, **59**, 171.

Reynaud, C.-A., Dahan, A., Anquez, V. and Weill, J.-C. (1991) The chicken D locus and its contribution to the immunoglobulin heavy chain repertoire. *Eur. J. Immunol*., **21**, 2661.

Romanoff, A.L. and Romanoff, A.J. (1949) *The avian egg*. Wiley, New York.

Romito, M., Viljoen, G.J. and Du Plessis, D.H. (2001) Eliciting antigen-specific egg-yolk IgY with naked DNA. *Biotechniques*, **31**, 670.

Rose, M.E., Orlans, E. and Buttress, N. (1974) Immunoglobulin classes in the hen's eggs: Their segregation in yolk and white. *Eur. J. Immunol*., **4**, 521–523.

Rutgeerts, P., D'Haens, G., Targan, S., Vasiliauskas, E., Hanauer, S.B., Present, D.H., Mayer, L., Van Hogezand, R.A., Braakman, T., DeWoody, K.L., Schaible, T.F. and Van Deventer, S.J. (1999) Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (inflixamab) to maintain remission to Crohn's disease. *Gastroenterology*, **117**, 761.

Sack, R.B. (1986) Antimicrobial prophylaxis of travellers' diarrhea: A selected summary. *Rev. Infect. Diseases*, **8**, S160.

Sandborn, W.J. and Hanauer, S.B. (1999) Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results and safety. *Inflamm. Bowel Dis*., **5**, 119.

Schade, R., Pfister, C., Halatsch, R. and Henklein, P. (1991) Polyclonal IgY antibodies from chicken egg yolk-an alternative to the production of mammalian IgG type antibodies in rabbits. *ATLA*, **19**, 403.

Schade, R., Staak, C., Hendrikson, C., Erhard, M., Hugl, H., Koch, G., Larsson, A., Pollmann, W., van Regenmortel, M., Rijke, E., Spielmann, H., Steinbush, H. and Straughan, D. (1996) The production of avian (egg yolk) antibodies: IgY. *ATLA*, **24**, 925.

Schmidt, P., Hafner, A., Reubel, G.H., Wanke, R., Franke, V., Losch, U. and Dahme, E. (1989) Production of antibodies to canine distemper virus in chicken eggs for immunochemistry. *J. Vet. Med*., **36**, 661.

Schrader, M., Temm-Grove, C.J., Lessard, J.L. and Jockusch, B.M. (1994) Chicken antibodies to rabbit muscle actin with a restricted repertoire of F-actin recognition. *Eur. J. Cell Biol*., **63**, 326.

Shelver, W.L., Larsen, G.L. and Huwe, J.K. (1998) Use of an immunoaffinity column for tetrachlorodibenzo-p-dioxin serum sample cleanup. *J. Chromatogr. B. Biomed. Sci. Appl*., **705**, 261.

Sharma, J.M. (1997) The structure and function of the avian immune system. *Acta. Vet. Hung*., **45**, 229.

Sharma, J.M. (1999) Introduction to poultry vaccines and immunity. *Adv. Vet. Med*., **41**, 481.

Shelver. W.L., Larsen, G.L. and Huwe, J.K. (1998) Use of an immunoaffinity column for tetrachlorodibenzo-p-dioxin sample cleanup. *J. Chromatogr. B Biomed. Sci. Appl*., **705**, 261.

Shimizu, M., Fitzsimmons, R.C. and Nakai, S. (1988) Anti-*E. coli* immunoglobulin Y isolated from egg yolk of immunized chickens as a potential food ingredient. *J. Food Sci*., **53**, 1360.

Shimizu, M., Nagashima, H., Sano, K., Hashimoto, K., Ozeki, M., Tsuda, K. and Hatta, H. (1992) Molecular stability of chicken and rabbit immunoglobulin G. *Biosci. Biotech. Biochem*., **56**, 270.

Shimizu, M., Nagashima, H. and Hashimoto, K. (1993) Comparative studies on molecular stability of immunoglobulin G from different species. *Comp. Biochem. Physiol*., **106B**, 255.

Shimizu, M., Nagashima, H., Hashimoto, K. and Suzuki, T. (1994) Egg yolk antibody (IgY) stability in aqueous solution with high sugar concentrations. *J. Food Sci*., **59**, 763.

Sim, J.S. and Nakai, S. (1994) *Egg Uses and Processing Technologies; New developments*. CAB international, Oxon, UK.

Sim, J.S., Nakai, S. and Guenter, W. (1999) *Egg Nutrition and Biotechnology*. CAB publishing, Oxon, UK.

Sim, J.S., Sunwoo, H.H. and Lee, E.N. (2000) Ovoglobulin IgY, In *Natural Food Antimicrobial Systems*, ed. Naidu, A.S. CRC Press, New York, p. 227.

Smith, D.J., King, W.F. and Godiska, R. (2001) Passive transfer of immunoglobulin Y to Streptococcus mutans glucan binding protein B can confer protection against experimental dental caries. *Infect. Immun*., **69**, 3135.

Stevenson, R.M.W., Flett, D. and Raymond, B.T. (1993) Enteric redmouth (ERM) and other enterobacterial infections of fish, In *Bacterial Diseases of Fish*, eds Inglis, V., Roberts, R.J. and Bromage, N.R. Blackwell Scientific Publications, Oxford, p. 80.

Sugita-Konishi, Y., Shibata, K., Yun, S.S., Hara-Kudo, Y., Yamaguchi, K. and Kumagai, S. (1996) Immune functions of immunoglobulin Y isolated from egg yolk of hens immunized with various infectious bacteria. *Biosci. Biotech. Biochem*., **60**, 886.

Sugita-Konishi, Y., Ogawa, M., Arai, S., Kumagai, S., Igimi, S. and Shimizu, M. (2000) Blockade of Salmonella enteritidis passage across the basolateral barriers of human intestinal epithelial cells by specific antibody. *Microbiol. Immunol*., **44**, 473.

Sun, S., Mo, W., Ji, Y. and Liu, S. (2001) Preparation and mass spectrometric study of egg yolk antibody (IgY) against rabies virus. *Rapid Commun. Mass Spectrom*., **15**, 708.

Sunwoo, H.H., Nakano, T., Dixon, T. and Sim, J.S. (1996) Immune responses in chicken against lipopolysaccharides of *Escherichia coli* and *Salmonella typhimurium*. *Poultry Sci*., **75**, 342.

Svennerholm, A.M., Holmgren, J. and Sack, D.A. (1989) Development of oral vaccines against enterotoxigenic *Escherichia coli*. *Vaccine*, **7**, 196.

Svendsen, L., Crowley, A., Ostergaard, L.H., Stodulski, G. and Hau, J. (1995) Development and comparison of purification strategies for chicken antibodies from egg yolk. *Lab. Anim. Sci*., **45**, 89.

Tacket, C.O., Losonsky, G., Link, H., Hoang, Y., Guerry, P., Hilpert, H. and Levine, M.M. (1988) Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. *New Eng. J. Med*., **318**, 1240.

Thalley, B.S. and Carroll, S.B. (1990) Rattlesnakes and scorpion antivenoms from the egg yolk of immunized hens. *Biotechnol*., **8**, 934.

Thorns, C.J., Sojka, M.G. and Chasey, D. (1990) Detection of a novel fimbrial structure on the surface of *Salmonella enteritidis* by using a monoclonal antibody. *J. Clin. Microbiol*., **28**, 2409.

Thorns, C.J., Sojka, M.G., McLaren, M. and Dibb-Fuller, M. (1992) Characterization of monoclonal antibodies against a fimbrial structure of *Salmonella enteritidis* and certain other serogroup D salmonellae and their application as serotyping reagents. *Res. Vet. Sci*., **53**, 300.

Tini, M., Jewell, U.R., Camenish, G., Chilov, D. and Gassmann, M. (2002) Generation and application of chicken egg-yolk antibodies. *Comp. Biochem. Physiol. A Mol. Integr. Physiol*., **131**, 569.

Tressler, R.L. and Roth, T.F. (1987) IgG receptors on the embryonic chick yolk sac. *J. Biol. Chem*., **262**, 15406.

Udhayakumar, V. and Muthukkaruppan, V.R. (1987) Protective immunity induced by outer membrane proteins of *Salmonella typhimurium* in mice. *Infect. Immun*., **55**, 816.

Verdoliva, A., Basile, G. and Fassina, G. (2000) Affinity purification of immunoglobulins from chicken egg yolk using a new

synthetic ligand. *J. Chromatogr. B. Biomed. Sci. Appl*., **749**, 233.

Vikinge, T.P., Askendal, A., Liedberg, B., Lindahl, T. and Tengvall, P. (1998) Immobilized chicken antibodies improve detection of serum antigens with surface plasmon resonance (SPR). *Biosens. Bioelectron*., **13**, 1257.

Wang, H.Y. and Imanaka, T. (1995) *Antibody Expression and Engineering*. American Chem. Soc., Washington, DC.

Warr, G.W., Magor, K.E. and Higgins, D.A. (1995) IgY: clues to the origins of modern antibodies. *Immunol. Today*, **16**, 392.

White, D.O. and Fenner, F.J. (1994) *Reoviridae*, In *Medical Virology*, Fourth Edition. Academic Press, New York, p. 522.

Wierup, M. (2000) The control of microbial diseases in animals: alternatives to the use of antibodies. *Int. J. Antimicrob. Agents*, **14**, 315.

Worledge, K.L., Godiska, R., Barrett, T.A. and Kink, J.A. (2000) Oral administration of avian tumor necrosis factor antibodies effectively treats experimental colitis in rats. *Dig. Dis. Sci*., **45**, 2298.

Yamanaka, H.I., Inoue, T. and Ikeda-Tanaka, O. (1996) Chicken monoclonal antibody isolated by a phage display system. *J. Immunol*., **157**, 1156.

Yokoyama, H., Peralta, R.C., Diaz, R., Sendo, S., Ikemori, Y. and Kodama, Y. (1992a) Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic *Escherichia coli* infection in neonatal piglets. *Infect. Immun*., **60**, 998.

Yokoyama, H., Peralta, R.C., Sendo, S., Ikemori, Y. and Kodama, Y. (1992b) 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*., **54**, 867.

Yokoyama, H., Umeda, K., Peralta, R.C., Hashi, T., Icatlo, F., Kuroki, M., Ikemori, Y. and Kodama, Y. (1998a) Oral passive immunization against experimental salmonellosis in mice using chicken egg yolk antibodies specific for *Salmonella enteritidis* and *S. typhimurium*. *Vaccine*, **16**, 388.

Yokoyama, H., Peralta, R.C., Umeda, K., Hashi, T., Icatlo, F.C., Kuroki, M., Ikemori, Y. and Kodama, Y. (1998b) Prevention of fatal salmonellosis in neonatal calves, using orally administered chicken egg yolk *Salmonella*-specific antibodies. *Am. J. Vet. Res*., **59**, 416.

Yolken, R.H., Leister, F., Wee, S.B., Miskuff, R. and Vonderfecht, S. (1988) Antibodies to rotavirus in chickens' eggs: a potential source of antiviral immunoglobulins suitable for human consumption. *Pediatrics*, **81**, 291.