A major milestone in man’s progress from primitive existence to civilized life is settled agriculture and domestication of livestock. Ever since the establishment of civilisation, humans have depended on animals for many requirements, such as that of food (milk, meat and egg), clothing (hide or wool), labour (pulling, carrying load) and security etc. Humans have consistently tried to improve the agriculture and domesticated animals to make them more useful for human welfare.

India’s agricultural area is vast with total arable and permanent cropland of 170 million hectares in 2003-2005. It has the second largest arable area in the world after the United States (MAP-European Communities, 2007). With nearly 12 percent of the world’s arable land, India is the world’s third-largest producer of food grains, the second-largest producer of fruits and vegetables and the largest producer of milk; it also has the largest number of livestock. Added to that is a range of agro climatic regions and agri-produce, extremely industrious farmers, a country that is fundamentally strong in science and technology, a government committed to Indian agriculture and an economy that is on the verge of double-digit growth. The sector supports an estimated 70 percent of the Indian population. Today, agriculture accounts for 14.2 percent of the country’s gross domestic product (Report of APCO Worldwide, 2011).

2.1. Livestock Sector in India: Livestock sector plays an important role in Indian economy and is an important subsector of Indian Agriculture. The contribution of livestock to Gross Domestic Product was 4.70% in 2004-05. This is the sector where the poor contribute to growth directly instead of getting benefit from growth generated elsewhere. The overall growth rate in livestock sector is steady and is around 4-5% and this has been achieved despite the fact that investment in this sector was not substantial. The ownership of the livestock is more evenly distributed with landless labourers and marginal farmers owning bulk of livestock. The progress in the sector
results in balanced development of the rural economy particularly in reducing the poverty amongst the weaker sections. India now occupies number one position in the world in respect of milk production and fourth position in egg production. Within the livestock sector, poultry has been the fastest growing sub-sector (GOI 2007).

2.2. The Poultry Industry: In the last few years the poultry industry has faced a number of challenges including the avian influenza outbreak in Asia that spread west throughout 2005 and early 2006. Despite this and other difficulties, poultry and eggs continue to be a hugely important source of animal protein, with poultry meat production, consumption and trade all increasing steadily since the late 1990s. Although the category of poultry covers many different species, including ducks, geese and ostriches, chicken and turkey are farmed for their meat much more than any other, with chicken alone accounting for over 85% of all poultry meat produced worldwide. The continuing steady rise in human population, particularly in Asia has helped poultry meat and egg consumption increase consistently over the last few years. The rise in popularity of poultry meat can also be attributed to its versatility, relative low cost in comparison to other meats and the acceptability of poultry meat to all religions (Pattison, 2007).

2.3. Poultry sector in India: The Indian Poultry Industry has transformed from small backyard farming to a well organized techno commercial industry. Between 1985 and 2005 poultry meat and egg production grew by about 12% and 5% per year, compared to an annual growth rate of 1.5 to 2.0% for beef, milk, mutton and lamb. At present with an average annual consumption of 1.5kg of poultry meat and 1.8kg of eggs (35-40 eggs) per person, exclusive of milk though, poultry meat and eggs contribute almost 50% to the per caput consumption of animal protein (Pica-Ciamarra and Otte, 2009). The significant step in poultry development has come from the initiatives taken by the private sector. The sector provides a great
employment opportunity. The government has now to play a limited role with reference to policies deal with the organized sector and to provide support to development of rural poultry. The productivity in both broilers and layers has improved massively due to the execution of good management practices, optimum nutrition and scientific breeding without compromising animal welfare. A clear example of such progress is the advances in poultry performance. Currently, the weight of modern chicken broilers is about 2700g at 42 days of age whereas, 50 years ago, commercial chickens of the same age would not have reached 600g at this age (Havenstein et al., 2003). Along with this progress came new challenges, including higher stocking densities, litter reutilization and health problems (Dekich, 1998; Duley-Cash, 2001). Such conditions have created the potential for pathogenic microbial colonization in birds (Cheema et al., 2003).

2.4. Poultry Diseases: The disease in poultry birds not only kills birds but also causes poor growth, poor hatchability, and poor egg production. Two types of losses may be from poultry diseases: direct and indirect. Direct loss includes value of the dead birds rearing and feeding costs up to the time of the death of poultry birds. The indirect loss may be due to poor growth rate and sick and less productive birds. An effective knowledge about containing poultry disease is crucial for successful poultry keeping. Severe disease often results in heavy mortality rate of poultry birds. In a few cases this results into the devastation of entire flock. The points to be considered in poultry business are: poultry disease; symptoms; their causes; the diagnosis; prevention of the diseases and their control. There are many poultry diseases and the important ones of them are: Parasitic; Bacterial; Viral; Fungal; Protozoal; Nutritional and Miscellaneous disease. (Agarwal, 1994).

2.5. Enteric Disorders in Poultry: Enteric disorders are one of the most important groups of diseases they affect poultry and are continuing to cause high economic losses in the many areas
worldwide due to increased mortality rates, decreased weight gain, increased medication costs and increased feed conversion rates. Several pathogens (Viruses, bacteria and parasites) are incriminated as possible causes of enteric disorders either alone (mono-causal), in synergy with different other microorganisms (multi-causal) or with non-infectious causes such as feed and/or management related factors (Table 1).

**Table 1: Some possible causes of enteric disorders in poultry**

<table>
<thead>
<tr>
<th>Non-infectious</th>
<th>Infectious</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feed</strong></td>
<td><strong>Viral agents</strong></td>
</tr>
<tr>
<td>Structure</td>
<td>Reo, Astro, Entero, Rota</td>
</tr>
<tr>
<td>Palatability</td>
<td>Coronavirus enteritis, HE</td>
</tr>
<tr>
<td>Energy content</td>
<td>ND, Influenza A</td>
</tr>
<tr>
<td>Pellet quality</td>
<td><strong>Bacterial agents</strong></td>
</tr>
<tr>
<td><strong>Management</strong></td>
<td><em>Salmonella, E. coli</em></td>
</tr>
<tr>
<td>Available feed space</td>
<td><em>Clostridia</em></td>
</tr>
<tr>
<td>Available water space</td>
<td><strong>Mycotic agents</strong></td>
</tr>
<tr>
<td>Distribution of feeders</td>
<td><em>Candida</em></td>
</tr>
<tr>
<td>Distribution of feeders</td>
<td><strong>Parasites</strong></td>
</tr>
<tr>
<td>Air quality</td>
<td>Coccidia, Histomon,</td>
</tr>
<tr>
<td>Temperature</td>
<td>Hexamitia, Ascaridia</td>
</tr>
<tr>
<td>Stocking density</td>
<td></td>
</tr>
</tbody>
</table>

Under field conditions, however, it is difficult to find out whether the true cause of enteric disorders in poultry is of infectious or non-infectious origin (Hafez, 2001). Several infectious agents like viruses, bacteria, fungus and parasites are involved in intestinal disorders. These agents can introduce and spread in poultry farms by different routes. At early days of age, the main disease problems are related to vertical and/or horizontal transmission of *Salmonella* and *E.coli* because of improper hatchery management or direct contact between infected and non-infected susceptible birds or through indirect contact with contaminated feed, water, equipment, environment and dust by ingestion or inhalation (Hafez, 2011).
2.6. **Salmonellosis in Poultry:** *Salmonella* infection is a problem of economic concern to all phases of poultry industry and also a source of food borne transmission of disease to humans. With the great expansion of poultry industry, the wide spread occurrence of Salmonellosis is considered as one of the most important bacterial diseases of poultry, even the survivor of *Salmonella* infection becomes the carrier for life and can become source of infection for other birds. This problem is major concern for breeders as they produce hatching eggs which carries these bacteria and resulting chicken are born with the infection and frequently die. *Salmonella* is a gram negative rod type of bacteria having known strains of more than 3000 but only few of them having concern in the poultry (Manoj, 2008). There are mainly two types of non motile avian *Salmonella* sp. namely *Salmonella gallinarum* and *Salmonella pullorum* that cause fowl typhoid and *Pullorum* disease respectively. Besides, motile *Salmonella* sp. (paratyphoid group) such as *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium cause Salmonellosis in chickens and have zoonotic significance (Hossain *et al.*, 2006).

2.7. **Transmission of Avian Salmonellosis:** *Salmonella* can be transmitted horizontally or vertically. Some predisposing factors like poor ventilation, overcrowding, high brooding temperature, nutritionally imbalance diet are also responsible for increasing the possibilities of infection. Generally *Salmonella* is having tendency to colonize in the lower part of the gut of chicken, Cloaca, which effects external opening of the oviduct. Therefore, during process of egg formation there might be chance of infection on the pores of the egg. There are evidences that *Salmonella* organism can become established in ova which results in vertical transmission of *Salmonella* through yolk. Once infected, other chickens become quickly infected by the horizontal transmission. This infection is further spread rapidly by the contaminated feed and water. It is very important to find out symptoms of *Salmonella* in early stage and start treatment because
young chicks are more susceptible for *Salmonella* infection (Manoj, 2008).

2.8. **Fowl Typhoid and Pullorum Disease**: Fowl typhoid and *Pullorum* disease, caused by *Salmonella enterica* subspecies *enterica* serovars Gallinarum biovars Gallinarum and Pullorum, respectively, are widely distributed throughout the world but they have been eradicated from commercial poultry in many developed countries. However, *S.gallinarum* has recently recurred in some European countries (Shah *et al.*, 2005). *Salmonella pullorum* remains in wild and game birds. *S. gallinarum* and *S. pullorum* are host adapted to avian species and are considered to cause a minimal zoonotic risk (Shivaprasad, 2000).

2.8.1. **Morphology and Staining**: They are non-motile, slender rod shaped Gram-negative organisms (Khan, 2004 and OIE Manual, 2010). On non-inhibitory media, colonies are small, round, glistening, dome shaped, smooth and 1 to 2 mm in diameter after 24-48 hours incubation (Pomery and Nagaraja, 1991). *Salmonella pullorum* produces smaller colonies than other *Salmonella* (OIE Manual, 2008).

2.8.2. **Growth Media**: *S. pullorum* and *S. gallinarum* both grow well on non selective media, but selective and enrichment media have been described that contain substances to inhibit the growth of extraneous organisms. Solid, Semisolid and broth can be employed. MacConkey agar, Deoxycholate citrate agar (DCA), Brilliant green agar and *Salmonella*-Shigella agar may be used as selective media (Doughlas *et al.*, 1998). MacConkey agar is inhibitory to non enteric organisms other than *Salmonella*, differentiates lactose fermenters from nonlactose fermenters. DCA is inhibitory to non enteric organisms. *S. gallinarum* colonies are 2-3mm in diameter, dome-shaped, with a central black spot. Brilliant green agar is inhibitory to coliforms and most *Proteus* isolates, useful for distinguishing enteric organisms colonies.
**Table 2: Characteristics of fowl typhoid and *Pullorum* disease**

<table>
<thead>
<tr>
<th>Points</th>
<th>Fowl Typhoid</th>
<th><em>Pullorum</em> Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hosts</strong></td>
<td>Birds (Primarily chickens and turkeys)</td>
<td>Birds (Primarily chickens and turkeys)</td>
</tr>
<tr>
<td><strong>Age Group</strong></td>
<td>Birds of all age (Most frequently in birds older than 3 months)</td>
<td>Birds under 6 weeks of age (acute form under 3 weeks of age)</td>
</tr>
<tr>
<td><strong>Transmission</strong></td>
<td>Horizontal and Vertical</td>
<td>Horizontal and Vertical</td>
</tr>
<tr>
<td><strong>Pathogenesis</strong></td>
<td>Faecal-oral/vertical</td>
<td>Faecal-oral/vertical</td>
</tr>
<tr>
<td></td>
<td>a) Carrier Septicemia</td>
<td>a) Carrier Septicemia</td>
</tr>
<tr>
<td></td>
<td>b) Systemic</td>
<td>b) Systemic</td>
</tr>
<tr>
<td><strong>Incubation Period</strong></td>
<td>Few days (4-5 days)</td>
<td>Few days (4-5 days)</td>
</tr>
<tr>
<td><strong>Course of infection</strong></td>
<td>Dependent upon:</td>
<td>Dependent upon:</td>
</tr>
<tr>
<td></td>
<td>-Breed of chicken</td>
<td>-Breed of chicken</td>
</tr>
<tr>
<td></td>
<td>-Nutritional and immune status</td>
<td>-Nutritional and immune status</td>
</tr>
<tr>
<td></td>
<td>-Virulence of strain</td>
<td>-Virulence of strain</td>
</tr>
<tr>
<td></td>
<td>-Management</td>
<td>-Management</td>
</tr>
<tr>
<td></td>
<td>-Age and sex of birds</td>
<td>-Age and sex of birds</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td>0-100% dependent upon factors mentioned above</td>
<td>The same – But generally low in adult fowl</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td>Sudden death, weakness, depression, diarrhoea, inappetance, respiratory distress, loss of weight anaemic and icteric mucous membrane and pallor of comb followed by cyanosis</td>
<td>The same – But no signs of anemia</td>
</tr>
<tr>
<td><strong>Pathological lesions</strong></td>
<td>Acute: Swelling of spleen, liver and kidney and peritonitis &amp; enteritis – anaemia</td>
<td>The same – But no signs of anaemia</td>
</tr>
<tr>
<td></td>
<td>Subacute &amp; chronic: discoloured swollen liver, necrotic foci on liver, spleen and myocardium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oophoritis, pericarditis, emaciation and anaemia</td>
<td></td>
</tr>
</tbody>
</table>

Source: PhD Thesis by Christensen (1996), KVL, Denmark
Salmonella from low, convex, pale red, translucent colonies of 1-2mm in diameter. *Salmonella pullorum* produces smaller colonies than other *Salmonella* (OIE Manual, 2008). Semisolid media made of heart infusion and agar can be used to observe motility. Selenite F broth and Brilliant green broth could be used as enrichment liquid media. Selenite F broth is inhibitory to coliforms but not *Proteus*, improved by addition of brilliant green. Selenite cysteine broth is more stable. Brilliant green broth or tetrathionate broth is inhibitory to coliforms and *Proteus*, but may also inhibit *S. pullorum/gallinarum*. Non inhibitory media include nutrient agar and blood agar, on which colonies are seen to be smooth, translucent, slightly raised and about 2mm in diameter. Broths include nutrient and heart infusion broth (OIE Manual, 2008).

### 2.8.3. Biochemical Properties: *

*Salmonella pullorum* can be differentiated from *S. gallinarum* by few biochemical characters. Arabinose, Dextrose, Galactose, Mannitol, Mannose, Rhamnose and Xylose are fermented by both the organisms with or without gas production. *S.gallinarum* ferments dulcitol, whereas *S. pullorum* does not. *S. pullorum* decarboxylates ornithine, whereas *S. gallinarum* does not. Decarboxylation of ornithine by *S. pullorum* is considered the single most dependable test for differentiation from each other (Shivaprasad, 2003).

### 2.8.4. Pathogenesis and Epidemiology

**(a) Prevalence:** Pullorum disease and fowl typhoid are worldwide in distribution. Although Pullorum disease is rare in commercial poultry in USA and Europe and perhaps in other parts of the world, it is still common in backyard poultry and in commercial level in developing countries. There is low incidence of fowl typhoid in USA, Canada and several European countries. Dramatic increase of fowl typhoid has been reported in South America and Africa. Reports on prevalence of *Pullorum* disease and fowl typhoid from many developing countries are available. Determination of prevalence based on isolation and
Identification record *Salmonella gallinarum* was found to be 4% in apparently healthy animals in North-eastern India (Battacharya *et al.*, 2004). The prevalence of *Salmonella pullorum* was higher in winter/spring than in other seasons. Thirty eight percent of positive reactor birds were found in broiler parent stock in India based on whole blood agglutination test (Asis *et al.*, 2001).

**(b) Natural Hosts and Age susceptibility:** *Salmonella enterica* serovars Gallinarum biovars *Pullorum/Gallinarum* are highly adapted to birds and several avian species are reported to be infected naturally and experimentally. Chickens are the natural host for *S. pullorum* and *S. gallinarum*. But outbreaks of *pullorum* disease and fowl typhoid were reported in other avian species also. Pullorum disease affects mainly chicks under 3 weeks of age and the initial indication is usually excessive numbers of dead in shell chicks and death immediately after hatching, sometimes it may appear in adult birds. Fowl typhoid frequently is referred to as a disease of adult birds but high mortality due to fowl typhoid has been recorded in young chickens also (Shivaprasad, 2003).

**(c) Pathogenesis:** The pathogenesis and pathogenicity of *Salmonella* depend on the invasive properties and the ability of the bacteria to survive and multiply within the cells, particularly macrophages (Humbert and Salvat, 1997). The bacteria infect and multiply within the cells of mononuclear phagocytic systems of the chicks and turkey. The principal site of multiplication of these bacteria is the digestive tract which may result in widespread contamination of the environment due to bacterial excretion through feces. Following invasion through the intestinal mucosa, cecal tonsils and Peyer’s patches, the organisms are taken up by macrophages, and through the blood stream and/or lymphatic systems, spread to organs rich in reticuloendothelial tissues, such as liver and spleen, which are the main sites of multiplication (Barrow *et al.*, 1994). In case of inadequate body defence mechanism, they may lead to second
invasion and be localized in other organs, particularly ovary, oviduct, myocardium, pericardium, gizzard, yolk sac and/or lungs (Barrow, 1993). Both biovars can cause septicemic infections, which may be acute or chronic, but unlike *S. pullorum*, *S. gallinarum* is capable of producing peracute-acute infection and haemolytic anaemia in both young and adults (Christensen, 1996). Haemolytic anaemia is induced by lipopolysaccharide and LPS-antibody complex. 

*S. pullorum* does not modify the RBC (Assoku and Penhale, 1974).

**Clinical Signs:** Clinical signs are typical of a septicaemic condition in poultry and include increased mortality and poor chicks hatched from infected eggs. Older birds show signs of anaemia, depression, laboured breathing and diarrhoea causing adherence of faeces to the vent. The highest mortality occurs in birds of 2-3 weeks of age. In older birds disease may be mild or inapparent. In breeding flocks reduced egg production and hatchability may be the only signs, and trans-ovarian infection resulting in infection of the egg and hatched chicks or poults is one of the most important transmission routes of the disease. Post-mortem signs of *Pullorum* disease in newly hatched chicks are those of peritonitis with generalized congestion of tissues and an inflamed unabsorbed yolk sac. Longer standing infections commonly lead to typhlitis with development of necrotic caecal casts and small necrotic foci in the liver, lungs and other viscera. Small lesions in the liver and spleen of *Pullorum*-infected birds may show a ‘white spot’ appearance that is not seen with Gallinarum; however, this lesion is not pathognomic. These *Salmonella* are very poor at colonisation and survival in the gastrointestinal tract is often indicative of later stages of clinical disease. Adult birds may develop misshapen or shrunken ovaries with follicles attached by pedunculated fibrous stalks. Variant strains of *S. Pullorum* do not normally cause clinical disease or may result in mild, nonspecific signs but may lead to seroconversion. In fowl typhoid, as well as generalised signs of septicaemia, the liver is usually enlarged, dark and friable with a distinctive coppery bronze sheen that may only
develop after exposure to air. The bone marrow is also often dark brown. Although clinical signs and post mortem findings of Pullorum disease and fowl typhoid may be highly suggestive of the conditions, they are not sufficiently distinct from other causes of septicaemia to be pathognomic. It is therefore necessary to confirm disease by isolation of the organisms. Serological tests can be used to establish the presence of the disease in a flock (OIE Manual, 2008).

(e) Morbidity and Mortality: Morbidity and mortality vary in chickens due to age, strain susceptibility, nutritional status, management and sex of the chickens (Shivaprasad, 2003). Mortality due to Pullorum disease varies from 0% to 100% whereas, that of 10% to 93% due to fowl typhoid in chicks depending on above factors and morbidity is often much higher than mortality (Shivaprasad, 2003). Prasanna and Paliwal (2002) conducted an experiment on Pullorum disease and fowl typhoid and they reported that 31% mortality due to fowl typhoid but no mortality was recorded due to Pullorum disease.

(f) Diagnosis: A tentative diagnosis of fowl typhoid and Pullorum disease can be made on flock history, clinical signs and post mortem lesions (Samad, 2005). But sometimes, fowl typhoid and Pullorum disease cannot be differentiated clinically and pathologically from other systemic diseases (Pomeroy and Nagaraja, 1991). Positive serologic findings can be of major value but not adequate for definitive diagnosis due to cross-reactions with other Salmonella (Shivaprasad, 2003). In addition, as there are no pathognomonic clinical signs and lesions of Pullorum disease and fowl typhoid, a definitive diagnosis of fowl typhoid and Pullorum disease can be made following isolation and identification of Salmonella pullorum and Salmonella Gallinarum, respectively (Shivaprasad, 2003 and Snoeyenbos, 1991). But the isolation and identification of Salmonella can be a very time-consuming and expensive process and includes the use of a number of selective and differential media (John, 1998) along with staining, morphological, biochemical and serological techniques.
(g) **Treatment:** A number of antibacterial agents reduce the morbidity and mortality of used to treat birds infected with Pullorum disease and/or fowl typhoid. Supply of 0.04% furazolidone in feed for consecutive 10 days is generally considered to be the best treatment (Shivaprasad, 2003). Luo and Chen (1987) used bacteria *Lactobacillus gallinaceus* (LG23, LG30 and LG160) in order to prevent and to cure the chickens from *Salmonella pullorum* infection and efficacy was superior to those of oxytetracycline and furazolidone. Batabyal *et al.*, (2003) isolated 298 isolates of *Salmonella gallinarum* from quails and drug sensitivity showed gentamycin (100%), ciprofloxacin (90%), streptomycin (90%), chloramphenicol (80%), nalidixic acid (80%) and cephalaxin (80%). The isolates were resistant to penicillin G and tetracycline (90%) followed by cloxacillin (30%). Sikder *et al.*, (2005) stated that experimental inoculation with isolated *S. gallinarum* caused typical signs of fowl typhoid, i.e. they were pathogenic to chicks as well as adult chickens. Antibiogram study suggested that the use of ciprofloxacin, cephalexin and kanamycin might be effective drugs to control fowl typhoid in the study areas. Plasmid profile analysis of the isolated *Salmonella* organism revealed that the isolates bearing multiple plasmids which might be the cause of various degree of antibiotic resistance.

2.9. **Paratyphoid Group – Foodborne illness in humans:** The various motile and non-host adopted highly invasive serotypes such as *Salmonella enteritidis* and *Salmonella typhimurium* are commonly referred to as paratyphoid *Salmonellae* or nontyphoid *Salmonellae*. Infection of poultry with *Salmonella* can arise primarily from nontyphoid infections, including *S. typhimurium*, *S. enteritidis* and *S. heidelberg*. Which are also associated with foodborne illnesses in human (D'Aoust, 1989). Young birds are more susceptible to systemic infections that can lead to increased early mortality; however, adult birds are more resistant and can potentially harbour *Salmonella* in their intestinal tract without showing any clinical signs (Brown *et al.*, 1976). These pathogens can be transmitted to poultry by several
Review of Literature

routes including faecal-oral, feed, water, insects, rodents, poult, humans, semen and transovarian route.

In recent decades, Salmonellosis (foodborne illnesses) has become a considerable burden to public health. Salmonella can cause a spectrum of pathological conditions such as acute gastroenteritis and bacteraemia in humans by mechanisms of colonization, invasion and penetration of the intestinal epithelium. The contamination of poultry meat products originates primarily from chickens infected with Salmonella during processing. In respect to this, S. enteritidis and S. typhimurium are of particular importance, since these pathogens can colonize the chicken host without causing discernible illness in the infected chicken. As per EU legislation the poultry meat has to be free from Salmonella on the market from 12/12/2010 onwards. If Salmonella is detected, the poultry industry needs to take measures to decrease the colonization of the birds and their environment. These include pre-harvest, harvest and post-harvest measures. Harvest measures are essentially hygienic during catching and transport, while post harvest measures include both hygienic measures and the application of decontaminating treatments on the meat. However, all carcass disinfectants are prohibited at present, thus decontamination is not an option. Therefore, prevention and monitoring during the live phase (pre-harvest phase) are of great importance (Chalghoumi et al., 2009).

2.10. Antibiotic Resistance in Poultry: Since the first report of Moore et al., (1946), it is generally known, that supplementation of poultry feed with antibiotic growth promoters (AGPs) improves performance of livestock. The effect of AGP on gut flora results in improvement of digestion, better absorption of nutrients, and a more stable balance in the microbial population. As consequence this is accompanied with reduced intestinal disorders. However, AGP can also increase the prevalence of drug-resistant bacteria. The modern production unit can produce market ready broiler chickens in less
than six weeks. This development arose from genetic selection, improved feeding and health management practices involving usage of antibiotics as therapeutic agents to treat bacterial diseases in intensive farming systems. They may also be used as prophylactic agents in the water of healthy birds and as growth promoters at sub-therapeutic concentrations in feed. Bacitracin, Chlortetracycline, tylosin, avoparcin, neomycin, oxytetracycline, virginiamycin and others are used for these purposes, sub-therapeutic dosing in feed increase the rate of weight gain and improve the efficiency of converting feed to meat. The recommended levels of antibiotics in feed were 5-10g kg\(^{-1}\) in the 1950s and have increased by ten to twenty folds since then. In many developing countries, majority of the antibiotics used in poultry is for treatment of infections. Antibiotics are also used to counteract the adverse consequences of stress responses (Apata, 2009).

The economic and health advantages of using antibiotics have revolutionized intensive poultry and livestock population (Apata 2009). In general, when an antibiotic is applied in poultry farming, the drug eliminates the sensitive bacterial strains, leaving behind or selecting those variants with unusual traits that can resist it. These resistant bacteria then multiply, increasing their numbers a million fold a day, becoming the predominant micro-organism in the population. Such bacteria transmit their genetically defined resistance characteristics to subsequent progeny of the strains and to other bacterial species via mutation or plasmid-mediated (Gould, 2008). According to WHO the resistance to antibiotics is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (Catry et al., 2003). Potential transfer of resistant bacteria from poultry products to human population may either occur through consumption or by handling meat contaminated with the pathogens (Van den Bogaard and Stobberingh, 2000). Once acquired, the resistant bacteria can colonize the human intestine and the genes coding resistance to antibiotics can be transferred to other bacteria belonging to the endogenous flora of humans, thereby jeopardizing
effective treatment of bacterial infections (De Leener, 2005). It is very important to monitor prevalence of resistance to antibiotics not only in human populations but also in animals in order to detect transfer of resistant bacteria or resistant genes from animal origin to humans and vice versa.

Fig.1: Sources of Antibiotics and Various Routes for the Transmission of Antibiotic Resistance from poultry and livestock to humans (Apata, 2009).
While the European Union, USA and Australia have recognized the serious consequences of antibiotic resistance from various areas of animal production for public health, there are large parts of Africa and Asia where we have little idea about antibiotic resistance. Therefore, researchers need to be focused on the development of resistance to antibiotics, incidence of antibiotic resistance in poultry, public health implications, strategy for the containment of the evolving bacterial resistances, as well as some alternative approach to antibiotic usage in poultry (Apata, 2009).

### 2.11. Alternatives to antibiotics in Poultry:

The recognition of the dangers of antibiotic resistance prompted the ban on sub-therapeutic antibiotic usage in Europe and the potential for a ban in the United States and many developed countries, there is increasing interest in using alternatives to antibiotics that have potential to reduce enteric diseases like Salmonellosis in poultry and subsequent contamination of poultry products. There are three basic strategies that can be employed to control Salmonella in poultry production throughout the live phase, such as preventive hygienic measures, vaccination and nutritional strategies or feed additives (Chalghoumi et al., 2009).

#### 2.11.1. Preventive Hygienic Measures (or) Sanitary Barrier:

Preventive Hygienic Measures typically involve establishing effective biosecurity and poultry house sanitation. This refers to an action plan designed to reduce the risk of introducing diseases or zoonotic agents into a flock. Because everything introduced into the poultry house is potentially contaminated, an effective control program must encompass several biosecurity measures. The starting point is to ensure that poultry breeding flocks, house, feed and litter are kept Salmonella free. Water also should be properly treated and free from pathogens (Doyle and Erickson, 2006). The European Food Safety Authority has reported specific hygiene measures to be followed for equipment, animals and people. However, such hygiene strategies are only partly effective for controlling pathogen colonization of the poultry.
house and thus complementary measures are essentially needed to control *Salmonella* efficiently (Mead, 2004).

**2.11.2. Vaccination:** Development of effective vaccines and disease control strategies needs better understanding of host immunity against disease producing agents. The poultry immune response to virulent and attenuated *Salmonella* strains is slight known. The importance of cell mediated immunity for tissues clearance (Barrow *et al.*, 2000) and systemic clearance (Farnell *et al.*, 2001) of virulent *Salmonella* strains is generally recognized. Vaccines have been designed with killed and live attenuated vaccine strains. The protective immunity provided by killed vaccines is inferior than that provided by live vaccines, because the killed vaccines mainly induces antibody production and is rapidly destroyed and eliminated from the host system (Vandeplas *et al.*, 2010). Killed vaccines generally fail to induce Tc cells (Nagaraj and Rajasekar, 1999) and secretory IgA responses, which are potentially important for protecting mucosal surfaces (Barrow *et al.*, 2000).

Although a number of different live *Salmonella* strains have been tested for their efficacy, only a few are registered and commercially available for use in poultry. Subunit vaccines, such as outer-membrane protein (Meenakshi *et al.*, 1999) or toxoid (Mishra and Sharma, 2001) also have been used in poultry. Prevention of the disease by vaccination is one of the promising alternatives to antibiotics; especially vaccination in breeder level is more convenient to control the disease transmission. Because, the maternal antibodies protect chicks from disease until they disappear. If any alteration in such temporary immunological protection through maternal antibodies like IgG, IgM and IgA results in immune suppression. Once the immunity goes down then the birds will easily acquire infections. Furthermore, the vaccination strategy cannot be applied in broiler production due to the short life span of the birds.
### 2.11.3. Feed Additives:

Preventive hygienic measures typically involve establishing effective farm-site biosecurity and poultry house sanitation protocols. Vaccination strategy cannot be applied in broiler due to the short life span of the birds. Since the ban on sub-therapeutic antibiotic usage in Europe, the use of feed additives is more and more accepted as a valuable way to combat *Salmonella* infection in poultry especially in broiler production (Chalghoumi *et al.*, 2009). Unlike antibiotics, alternatives such as organic acids, prebiotics, probiotics, symbiotics, competitive exclusion and bacteriophages have been designed partly to reduce the intestinal colonization and subsequent faecal excretion of *Salmonella*.

Passive immunization by oral administration of hen egg yolk antibody (IgY) is an emerging and promising nutritional strategy that may server to control *Salmonella* in poultry industry particularly in broiler chickens. Maternal antibodies transferred from the yolk to the chick prevent *Salmonella* colonization (Hassan *et al.*, 1996). This fact led to the hypothesis that feeding these antibodies to hatchlings would provide passive immune protection. Several researchers have investigated how hyper-immunized hens can produce pathogen-specific antibodies in large quantities in eggs (Schade *et al.*, 2005).

### 2.12. Avian Egg:

The avian egg is an important source of nutrients, containing all of the proteins, lipids, vitamins, minerals and growth factors required by the developing embryo, as well as a number of defence factors to protect against microbial infections mainly infections of bacterial and viral origins. Moreover, eggs are now understood to contain substances with biological functions beyond basic nutrients, and extensive research has been undertaken to identify and characterize these biological active components (Kovacs-Nolan *et al.*, 2005).

### 2.13. Avian Egg Formation:

Under modern husbandry condition, 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 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 reproductive system of the hen (Fig. 2) 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. 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 wall is 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 into the oviduct where the yolk is enveloped in albumen and the shell. It takes 24-27 hours for this development.

![Diagram of the reproductive system of the Hen: ovary and oviduct.](image)

**Fig. 2: The reproductive system of the Hen: ovary and oviduct (Adapted from Kovacs-Nolan and Mine, 2004).**
Table 3: Chemical composition of Hen Eggs

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% (w/v)</th>
<th>Major components</th>
<th>Relative % (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg shell (including shell membrane)</td>
<td>9.5</td>
<td>Inorganic salts 91.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteins 6.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water 1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipids 0.03</td>
<td></td>
</tr>
<tr>
<td>Egg white</td>
<td>63.0</td>
<td>Water 87.87 - 89.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteins 9.7 - 10.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carbohydrates 0.4 – 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipids 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ash 0.5 – 0.6</td>
<td></td>
</tr>
<tr>
<td>Egg yolk</td>
<td>27.5</td>
<td>Water 46.3 – 51.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipids 32.0 – 35.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteins 15.7 – 16.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carbohydrates 0.2 – 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ash 1.1</td>
<td></td>
</tr>
</tbody>
</table>

Modified from Kovacs-Nolan (2005)

Fig.3: Components of Eggs (http://www.chemicalbiological.net/the egg.html)
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. The hen normally starts laying at 16-26 weeks of age. Egg consists of approximately 9.5% eggshell (including shell membrane), 63% albumen and 27.5% yolk (Cotterill and Geiger, 1977). The main components are water (75%), proteins (12%) and lipids (12%) as well as carbohydrates and minerals (Li-Chan et al., 1995). 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. Many diverse biological functions have been attributed to egg components, in which the antimicrobial activity is an important one. The egg shell and shell membrane physically block invading microorganisms, differing in viscosities and pH values in the egg white inhibit bacterial proliferation.

2.14. Egg Yolk: The major constituents of the solid mater of yolk are proteins and lipids, present mainly in the form of lipoproteins (Li Chan et al., 1995). 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 (Burely 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 egg yolk antibody or IgY (Li Chan et al., 1995).
Table 4: Chemical composition of Egg Yolk

<table>
<thead>
<tr>
<th>Constituents</th>
<th>% (W/V)</th>
<th>Major Components (relative %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>15.7 – 16.6</td>
<td>Apovitellenin (I-VI) (37.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipovitellin apoproteins (40.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α- Lipovitellin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β- Lipovitellin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Livetins (9.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α- Livetin (serum albumin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β- Livetin (α2 glycoprotein)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ-Livetin (γ-globulin)(IgY)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phosvitin (13.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biotin-binding protein (trace)</td>
</tr>
<tr>
<td>Lipids</td>
<td>32.0-35.0</td>
<td>Triglycerol (66%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phosphatidylcholine (24%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phosphatidylethanolamine (2.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lysophosphatidylcholine (0.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sphingomyelin (5.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cholesterol (5.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others (1.0%)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.2-1.0</td>
<td>-</td>
</tr>
<tr>
<td>Ash</td>
<td>1.1</td>
<td>-</td>
</tr>
</tbody>
</table>

Adapted from Kovac-Nolan et al., (2004)

2.15. Avian Egg Antibodies - Avian immune system: Like in mammals, various mechanisms have been developed in birds to protect them from invading microorganisms and foreign substances. The bird’s immune system consists of primary lymphoid organs and secondary lymphoid organs. Primary organs are the thymus, located in the neck along the jugular vein, and the bursa of Fabricus, located adjacent to the cloaca. Secondary organs are the spleen, bone marrow, Harderian gland, caecal tonsils, and organized lymphoid tissues associated with mucosal surface (MALT), including bronchial associated lymphoid tissues (BALT), gut associated lymphoid tissues (GALT), conjunctival associated lymphoid tissues (CALT) and lymphoid nodules. There is also a lymphatic circulatory system of vessels and
capillaries that transport lymph fluid through the bird’s body and communicate with the blood supply (Davison et al., 2008). Functionally, the bird’s immune system can be divided into two parts: one innate, but non-specific, while the other part is acquired and specific. The acquired immune system is characterized by specificity, heterogeneity and memory. This system is further divided into cellular branch and non-cellular branch (humoral).

The non-cellular branch includes immunoglobulins and the cells which produce them. Antibodies are specific for the foreign material (antigen) to which they attach. The cells which produce antibodies are the B-lymphocytes. These cells are produced in the embryonic liver, yolk sack and bone marrow. The B-cells respond by producing antibodies after day 5 following exposure. The lag period occurs because the B-cells must be programmed and undergo clonal expansion to increase their numbers. If the chicken is exposed to a second time to the same antigen, the response is quicker and a much higher level of antibody production occurs (memory). The cellular branch of the bird’s immune system includes all the cells that react with specificity to antigens, except those associated with antibody production.

2.16. Antibodies Biosynthesis: Three immunoglobulin classes, which are distinguishable in concentration, structure, and immunochemical function, are found in birds: IgA, IgM and IgY. The IgA and IgM are similar to mammalian IgA and IgM in molecular weight, structure and electrophoretic mobility. 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 concentration of IgY, IgA and IgM have been reported to be 5.0, 1.25 and 0.61mg/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 (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 concentration of around 0.15 and 0.7 mg/ml, respectively, whereas the yolk may contain from 5 to 25 mg/ml of IgY (Li et al., 1997). Mammalian equivalents of IgE and IgD have not been identified in chickens (Sharma, 1997).

**Fig. 4: Translocation of IgY, IgA and IgM from the Blood to Egg**

During egg formation, IgY (blue) is transferred from the blood to the egg yolk through receptors specific for IgY translocation. IgA (green) and IgM (purple) are later deposited into the egg white in the oviduct. Adapted from Hatta et al., (2008), source: Kovacs-Nolan and Mine (2012).
2.17. **Structure of Immunoglobulin Y (IgY):** IgY has a molecular mass of ~180kDa which is heavier than that of mammalian IgG (~150kDa). The general structure of the IgY molecule consists of two identical heavy (H) chains and two identical light (L) chains, which are linked by disulfide bridge. The light chain of IgY consists of one variable (V1) and one constant domain (C1), like mammalian IgG. But, intra-chain disulfide linkage between the V\_L region and C\_L region of L-chain, which stabilizes the structure of the mammalian IgG L-chain is absent in the IgY L-chain and thus intra-molecular forces of IgY are weaker than those of mammalian IgG (Shimizu *et al.*, 1993). The heavy chain of IgY contains one variable domain (V\_H), four constant domains (C\_H\_1; C\_H\_2; C\_H\_3 and C\_H\_4), unlike mammalian IgG which has three constant domains (C\_H\_1; C\_H\_2 and C\_H\_3).

![Structure of IgG and IgY](Fig. 5: Structure of IgG and IgY (Kovacs-Nolan, 2004))

In the heavy chain of IgG, the C\_H\_1 and the C\_H\_2 domains are separated by a hinge region, which gives considerable flexibility to the Fab fragment (the portion which contains the antigen-binding activity). In contrast, the heavy chain of IgY does not have a hinge region, but there are regions (potential switch regions) near the boundaries of the C\_H\_1-C\_H\_2 and C\_H\_2-C\_H\_3 domains that contain proline...
and glycine residues. These regions have the potential to confer limited flexibility on the molecule. Comparisons of C-domain sequences in IgG and IgY have shown that the C\textsubscript{H2} and C\textsubscript{H3} domains of IgG are the equivalents of the C\textsubscript{H3} and C\textsubscript{H4} domains of IgY, respectively. The equivalent of C\textsubscript{H2} domain of IgY is absent in the heavy chain of IgG. The content of β-sheet structure in C domains of IgY is lower than that of mammalian IgG; therefore, the conformation of IgY domains is more disordered in comparison to mammalian IgG. As for IgG, the Fc part of IgY is the site of most biological effector functions. It contains two carbohydrate side-chains, in contrast to only one in IgG (Fig-2).

**Table 5: Comparison of mammalian IgG and Chicken IgY**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mammalian IgG</th>
<th>Chicken IgY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Sampling</td>
<td>Invasive</td>
<td>Non-invasive</td>
</tr>
<tr>
<td>Source of Antibody</td>
<td>Blood Serum</td>
<td>Egg Yolk</td>
</tr>
<tr>
<td>Antibody Amount</td>
<td>200mg/bleed (40ml Blood)</td>
<td>50-100mg IgY/egg (300 eggs/year)</td>
</tr>
<tr>
<td>Frequency of Collection</td>
<td>Every two weeks</td>
<td>Every Day</td>
</tr>
<tr>
<td>Quantity of antibody/year</td>
<td>5200mg</td>
<td>22,500mg</td>
</tr>
<tr>
<td>Amount of Specific Antibody</td>
<td>~5%</td>
<td>~2-10%</td>
</tr>
<tr>
<td>Protein A/G Binding</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Interference with mammalian IgG</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Interference with rheumatoid factor</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Activation of mammalian complement</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Adapted from Schade et al., 2005; Source: Xu et al., 2011

2.18. **Physiochemical Properties of IgY**: Polson et al., (1980) reported that the isoelectric point of IgY is lower than that of IgG. It is in the range of 5.7 to 7.6, whereas that of IgG lies between 6.1 and 8.5. Since the Fc fragment (the most hydrophotic moiety of the antibody molecule) of the IgY is bigger than that of the IgG, the IgY molecule is more hydrophobic than IgG molecule (Davalos et al., 2000).
**Table 6: Earliest Major Milestones in the Study of IgY**

<table>
<thead>
<tr>
<th>Year</th>
<th>Author(s)</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1892</td>
<td>Ehrlich</td>
<td>Studied transfer of passive immunity from mother to offspring</td>
</tr>
<tr>
<td>1893</td>
<td>Klemperer</td>
<td>Discussed transfer of passive immunity in the fowl</td>
</tr>
<tr>
<td>1899</td>
<td>Gross</td>
<td>Noted presence of a water soluble protein in yolk</td>
</tr>
<tr>
<td>1900</td>
<td>Osbourne and Campbell</td>
<td>Isolated “a large amount of protein” from yolk and identified it as vitellin</td>
</tr>
<tr>
<td>1903</td>
<td>Ewing</td>
<td>First used fowl precipitins in blood identification</td>
</tr>
<tr>
<td>1908</td>
<td>Plimmer</td>
<td>Isolated water soluble protein in yolk and named it livetin</td>
</tr>
<tr>
<td>1910</td>
<td>Sutherland</td>
<td>Used fowl precipitins on a large scale in blood identification</td>
</tr>
<tr>
<td>1918</td>
<td>Hektoen</td>
<td>Studied anti-human precipitins in chicken serum</td>
</tr>
<tr>
<td>1928</td>
<td>Kay and Marshall</td>
<td>Isolated livetin fraction in a relatively pure form, but noted signs of heterogeneity</td>
</tr>
<tr>
<td>1932</td>
<td>Jukes and Kay</td>
<td>Determined that IgY and chicken IgG are either closely related or identical</td>
</tr>
<tr>
<td>1949</td>
<td>Shepard and Hottle</td>
<td>Isolated 3 proteins from livetin fraction using electrophoresis</td>
</tr>
<tr>
<td>1950</td>
<td>Shmittle</td>
<td>Detected antibodies to Newcastle disease in egg yolk</td>
</tr>
<tr>
<td>1957</td>
<td>Martin <em>et al.</em>,</td>
<td>Isolated the 3 livetins and named them α-, β-, γ-livetin</td>
</tr>
<tr>
<td>1980</td>
<td>Polson <em>et al.</em>,</td>
<td>Developed the Polyethylene glycol (PEG) method for IgY purification</td>
</tr>
</tbody>
</table>

Source: PhD Thesis by Edward A. Charter (1993), the University of British Columbia,
Table 7: Comparison of rabbit and chicken polyclonal antibody yield during a two-week period following the second immunization

<table>
<thead>
<tr>
<th></th>
<th>Rabbit</th>
<th>Chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Method of sampling</td>
<td>Bleeding (20mL/week)</td>
<td>daily collecting of eggs</td>
</tr>
<tr>
<td>Sample volume (in 2 weeks)</td>
<td>40mL of blood</td>
<td>14 eggs = 210mL of egg yolk*</td>
</tr>
<tr>
<td>Amount of total antibodies</td>
<td>200mg</td>
<td>1120mg**</td>
</tr>
<tr>
<td>Amount of specific antibodies</td>
<td>5% (10mg)</td>
<td>2–10% (22.4–112mg)</td>
</tr>
<tr>
<td>Rabbits/chicken – total***</td>
<td>5–6</td>
<td>1</td>
</tr>
<tr>
<td>Rabbits/chicken – specific****</td>
<td>2-11</td>
<td>1</td>
</tr>
<tr>
<td>Presence of other Ig</td>
<td>IgM, IgA, IgE</td>
<td>None</td>
</tr>
</tbody>
</table>

*average volume of egg yolk is 15mL; **average amount of IgY is 80mg per one egg yolk; ***No. of rabbits that produce an equal amount of total antibodies as one chicken in a two-week period; ****No. of rabbits that produce an equal amount of specific antibodies as one chicken in a two-week period

Adopted from Mojca Narat, 2003

2.19. Stability of IgY

(a) pH stability: Extensive researchers have studied the stability of IgY to acid and alkali under various conditions. It has been found that the activity of IgY was decreased at pH 3.5 or lower and almost completely lost with irreversible change at pH 3 (Shimizu et al., 1993). Similar results were reported by Losch et al., (1986), Hatta et al., (1993) and Lee et al., (2002b). Rapid decrease of the IgY activity at low pH(s) indicated conformational changes and damage in the Fab portion including the antigen-binding site. The activity of IgY did not change under alkali condition at the pH 11 and it was found that the complete loss at pH 12 or higher (Shimizu et al., 1993). Many of the recent researches were presented the similar results.
(b) **Proteolysis stability:** IgY is relatively resist the trypsin or chymotrypsin attach and fairly resistance to pepsin digestion. It has been reported that 39% and 41% of the activity remained after 8 hours incubation with trypsin or chymotrypsin respectively. But, the complete loss of activity was observed following pepsin digestion (Hatta *et al.*, 1993). The stability of IgY against pepsin appears to be highly dependent on pH and the enzyme/substrate ratio. At pH 5 or higher, IgY was fairly resistant to pepsin and retained its antigen-binding and cell-agglutinating activities. However, at pH 4.5 or below, both activities were lost. Similar results have been reported by many researchers.

(c) **Temperature stability:** IgY has been treated at various temperature for different periods of time. The binding activity of IgY with antigen decreased with increasing temperature and heating time. According to Shimizu *et al.*, (1992) and Hatta *et al.*, (1993), IgY is stable at temperature ranging between 60°C and 70°C. The activity of IgY decreased by heating for 15 minutes at 70°C or higher and IgY denatured seriously when thermally treated at temperature higher than 75°C (Chang *et al.*, 1999).

2.20. **Chicken Egg Yolk antibody (IgY) production:** In 1893, Klemperer first described an experiment in which he demonstrated that the immunization of a hen resulted in the transfer of specific antibodies into the egg yolk. For a long time there was no scientific application for this knowledge, but when animal welfare became a matter of serious ethical concern for the scientific community, the results of Klemperer gained interest. In Particular, this development was initiated by the work of Russell and Bruch and the publication in 1959 of *The Principles of Humane Experimental Technique*. In the subsequent 20 years, more and more researchers recognised the importance of Klemperer’s results. Since the 1980s, egg yolk antibodies have found a broader application.
In 1996 an European Centre for the Validation of Alternative Methods (ECVAM) workshop recommended the use of IgY instead of mammalian IgG, in order to minimise pain caused by invasive antibody sampling. This workshop also provided information about practical aspects of the rearing of laying hens, immunization protocols, the use of adjuvants and IgY extraction methods. Now, the 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 100-150mg/egg (Rose et al., 1974). Therefore, an immunized hen yields more than 40g of IgY per year through eggs, it is equivalent to that from 40 rabbits.

2.20.1. Chicken as a Laboratory Animal: European Centre for the Validation of Alternative Methods (ECVAM) workshop (1996) has recommended some basic needs to use chicken as a laboratory animal. A basic requirement for the use of chickens is the availability of housing conditions which favours species-specific behaviour. Housing chickens in cages, under laboratory conditions is advantageous, in that the chickens can be readily located and their health can be easily monitored. Antibodies can be produced by using chickens bred for commercial egg production as well as those which have been bred free from specific pathogens (SPF chickens). Getting adult SPF chickens are relatively difficult; therefore they have to be raised in the laboratory. Commercial laying chickens are not only cheaper to purchase, but they can also be obtained just before they come into production, thereby further reducing the costs associated with antibody production. But, the advantage of using SPF chickens over commercial egg layers is that getting higher antibody titres against target antigen. Another important consideration is the egg-laying capacity of the chicken, and the possible factors which may affect it. One such factor could be immunization using Freund’s
complete adjuvant (FCA), or the antigen itself. According to some reports, FCA does not influence egg production.

**Recommendations (ECVAM workshop - 1996)**

1. Chickens should be kept under conditions which encourage natural behaviour. Research on environmental enrichment should be supported, so that housing conditions can be further improved.

2. It is preferable to keep chickens indoors, and to restrict entrance to chicken houses to authorised personnel only. Such personnel should have no contact with commercially maintained poultry.

3. For Scientific purpose (laboratory work), conventional housing (cages, with groups of at least two hens) should be used.

4. When antibodies are to be used for therapeutic purpose, the use of SPF chickens is compulsory.

5. Although chicken strains used commercially for egg production give an acceptable antibody response, it is preferable to use inbred strains in order to induce higher antibody responses.

6. It is preferable to immunize chickens before they begin to produce eggs, because the stress induced by handling then can have an adverse effect on egg production, as can the nature of the antigen or adjuvant used.

7. With respect to protocols for chicken immunization, recommendations about the adjuvant, antigen dose, injection site, volume and frequency, immunization intervals and the period for which chickens should be used are tabulated below (Table 8)

8. The term “IgG” should not be used for chicken antibodies, since it does not conform to our current knowledge on antibody structure.
9. A detailed and careful comparison of the different methods for purifying IgY should be undertaken.

10. A standard method should be established for determining IgY concentration.

Table 8: Recommendation relating to chicken Immunization protocols

<table>
<thead>
<tr>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant</td>
</tr>
<tr>
<td>Freund's incomplete adjuvant, specol, lipopeptide (Pam3-Cys-Ser-Lys)_4; 250µg</td>
</tr>
<tr>
<td>Antigen dose</td>
</tr>
<tr>
<td>10ng – 1mg (preferably 10-100µg)</td>
</tr>
<tr>
<td>Injection site</td>
</tr>
<tr>
<td>Intramuscular (field studies; young laboratory chickens)</td>
</tr>
<tr>
<td>Subcutaneous (older laboratory chickens)</td>
</tr>
<tr>
<td>Injection volume</td>
</tr>
<tr>
<td>&lt;1ml</td>
</tr>
<tr>
<td>Injection frequency</td>
</tr>
<tr>
<td>2-3 times; boosters during laying period</td>
</tr>
<tr>
<td>Vaccination interval</td>
</tr>
<tr>
<td>4-8 weeks</td>
</tr>
<tr>
<td>Use of Chickens</td>
</tr>
<tr>
<td>Entire laying period (about 1 Year)</td>
</tr>
</tbody>
</table>

2.20.2. Immunization of hens: Specific IgY development and production can be attained by immunizing laying hens with the target antigen. However, the consequential immune response of the immunized hens cannot be very predictable. Mainly five factors influence this response: the antigen (dose and molecular weight), the type of adjuvant used, the route of application, the immunization frequency, and the interval between immunizations (Schade et al., 1996).

(a) Antigen: The immune response is triggered by contact of the organism with antigen, which is a structure that is recognized by the immune system as foreign (non-self). The dose of antigen influences significantly the immune response and the antibody titer that is evoked. Too much or too little antigen may induce suppression, sensitization, tolerance or other unwanted immunomodulation (Hanly et al., 1995). Behn et al., (1996) have reported that best results observed when immunizing hens with 0.1mg of mouse IgG, instead of
1.0mg. The injection of antigen dose ranging from 10µg to 1mg has shown good antibodies responses (Schwarzkopf et al., 2000; Mahn, 1998). Basically for each antigen, various concentration have to be tested since the type of antigen should also be considered. Antigen can be presented to the immune system as complex multiantigens (e.g., bacteria, viruses and parasites) or as single antigens (e.g., proteins or polysaccharides) (Leenars et al., 1996). Proteins are recognized to be the most efficient immunogens due to the polymorphism of their structure and the difference existing between species and individuals (Goldsby et al., 2003). Peptides (molecular weight >10kDa) can also be used as antigen, but they should be couple to carriers (Adjuvant) (Schade et al., 2005). Polysaccharides antigens are efficient too. However, lipid and nucleic acids are not potent immunogens unless they are coupled to proteins or Polysaccharides (Goldsby et al., 2003).

(b) Adjuvant: The induction of high and sustainable egg yolk antibody titre reclaims the use of adjuvant. There are more than 100 known adjuvants, which differ in their chemical characteristics, their efficacy in stimulating the immune system, and their secondary side-effects. Freund’s complete adjuvant (FCA) remains the most effective adjuvant for antibodies production in laboratory animals. In mammals, the use of this adjuvant leads systematically to severe inflammation at the injection site. In birds, the use of FCA does not seem to result in the same severe lesions as in mammals. To avoid an eventual local tissue reaction, the Freund’s incomplete adjuvant (FIA), which is the most effective substitute found to date, becomes now the most commonly used adjuvant to produce egg yolk antibody. Since FIA is less efficient than FCA, some investigators preferred the use of a combination of the two adjuvants: FCA for the first immunization and FIA for the booster immunizations (Chalghoumi et al., 2008). In these studies good results were achieved and no adverse side-effects were reported.
(c) Route of application: The most common route for antigen injection in hens for IgY production is the intramuscular route. Injection is usually performed in the breast muscle (Schade et al., 2005). Some authors inject antigen in the leg, but the general recommendation states that intramuscular injection in the leg should be avoided, since it can lead to lame. Chicken can also be injected subcutaneously in the neck. With very young animals, it may be preferable to inject intramuscularly into the breast muscle, because subcutaneous injection is more difficult to perform and can therefore cause more distress (Schade et al., 1996).

(d) Immunization frequency and interval between immunizations: The total numbers of immunizations required depend on the type and dose of the antigen as well as the adjuvant employed. At least two immunizations have to be given. Yolk antibody titres should be checked 14 days after the last immunization. If antibodies titres begin to decrease, booster immunizations can be given during the laying period to maintain production of high levels of specific antibodies up to year (Schade et al., 1996). The success of an immunization protocol depends also on the interval between the first and second and subsequent immunizations. Often reported interval is two to four weeks (Tini et al., 2002).

2.20.2. Purification of IgY: Lipids and proteins are the major constituents of egg yolk. The lipid fraction, including triglycerides, phospholipids, and cholesterol, constitutes approximately one third of the yolk. Proteins consist 15 to 17% of the yolk, which can be separated by centrifugation into two main fractions, the granule (precipitate on centrifugation) and the plasma (clear fluid supernatant on centrifugation) (Stadeklman et al., 1977). Plasma is about 78% of the total yolk proteins and composed of 86% of LDL and 14% of livetins (McCully et al., 1962). Livetins are water soluble, lipid-free globular glycoproteins, which are divided into three classes: α-, β-, and γ-livetin. According to (Bernardi et al., 1960) the relative
A proportion of the three livetins in the yolk is 2:5:3, respectively. IgY is the predominant fraction of γ-livetin (Kovacs-Nolan et al., 2005). Separation of IgY requires therefore the removal of the lipoprotein and the recovery of the water-soluble fraction (WSF) followed by purification of the IgY from other livetins (Polson et al., 1980). The removal of lipoproteins and the recovery of the WSF can be achieved by several methods, as presented in Table 9.

These various IgY crude extraction methods give different results towards recovery and purity. Akita and Nakai (1993) compared the water dilution methods to other methods such as polyethylene glycol, dextran sulphate, and alginates methods in terms of yield, purity and activity of IgY. The water dilution method yielded IgY in the highest level (91%), purity (31%) and with similar activity to that obtained by using other methods. Deignan et al., (2000) evaluated the dextran sulphate, the phosphotungstic acid, the polyethylene glycol and the isopropanol-aceton methods. The dextran sulphate, the phosphotungstic acid methods were both the better methods with regard to IgY recovery, followed by Polson method (Polson et al., 1985). The use of isopropanol-aceton method gave the lowest recovery. The choice of suitable IgY extraction method is mainly influenced by the quality of extraction (preservation of antibodies activity, purity and recovery of antibodies), scale of extraction (laboratory or industrial), cost effectiveness and technology. For example a wide use of IgY in food application, a large-scale production of IgY with high recovery and purity is necessary. Such a separation process should be simple, economical and requiring few chemicals. In view of these requirements, the water dilution method appears the most appropriate technique. The recovery of the WSF can be followed by an additional step to separate IgY (γ-livetins) from the other water-soluble proteins (α- and β-livetins), and the remaining LDL. Three kinds of separation can be used to eliminate these contaminants: precipitation, chromatography and filtration. Several of these techniques could be included in one total clean-up procedure (Chalghoumi et al., 2009).
Table 9: Methods of Purifying IgY

<table>
<thead>
<tr>
<th>Purification Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Precipitation and extraction</strong></td>
<td></td>
</tr>
<tr>
<td><em>Polyethylene glycol (PEG)</em></td>
<td>Polson <em>et al.</em>, 1980, and Akita and Nakai, 1993</td>
</tr>
<tr>
<td><strong>Dextran sulphate</strong></td>
<td>Jensenius <em>et al.</em>, 1981 and Akita and Nakai, 1993</td>
</tr>
<tr>
<td><strong>Ethanol</strong></td>
<td>Polson <em>et al.</em>, 1985, Hatta <em>et al.</em>, 1988</td>
</tr>
<tr>
<td><strong>Ammonium sulphate</strong></td>
<td>Akita and Nakai, 1992 and Svendsen <em>et al.</em>, 1995</td>
</tr>
<tr>
<td><strong>Chloroform</strong></td>
<td>Polson, 1990</td>
</tr>
<tr>
<td><strong>Dextran blue</strong></td>
<td>Bizhanovv and Vyshniausskis, 2000</td>
</tr>
<tr>
<td><strong>Caprylic acid</strong></td>
<td>McLaren <em>et al.</em>, 1994 and Svendsen <em>et al.</em>, 1995</td>
</tr>
<tr>
<td><strong>Propanol</strong></td>
<td>Bade and Stegemann, 1984</td>
</tr>
<tr>
<td><strong>Natural gums, k-carrageenan</strong></td>
<td>Hatta <em>et al.</em>, 1990 and Chang <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><strong>Sodium alginate</strong></td>
<td>Hatta <em>et al.</em>, 1988 and Chang <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><strong>Xanthan gum</strong></td>
<td>Akita and Nakai, 1993</td>
</tr>
<tr>
<td><strong>Carboxymethylcellulose (CMC)</strong></td>
<td>Chang <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><strong>Pectin</strong></td>
<td>Chang <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><strong>Chromatography</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Hydrophobic interaction chromatography</strong></td>
<td>Hassl and Aspock, 1988</td>
</tr>
</tbody>
</table>


2.20.3. Storage stability of IgY: The stability of IgY during storage is reasonably good under specified conditions. IgY solutions may be stored at 4 °C for periods ranging from months to a few years provided 0.02% NaN₃, 0.03%w/v thimerosal or 50 µg/ml gentamicin has been added to retard microbial growth (Schade *et al.*, 2005). Dried IgY
preparations were stored for five to ten years at 4 °C without significant loss of antibody activity and the preparations also retained activity for six months at room temperature and for one month at 37°C (Larsson et al., 1993). While lyophilization minimizes bacterial growth and maintains better structural integrity of purified IgY, spray-drying is more economical (Yokoyama et al., 1992). Collectively, these unique biological attributes make IgY an effective natural food antimicrobial system and immunotherapeutic agent (Xu et al., 2011).

2.21. Mode of Action through which IgY counteracts pathogens:
The exact mechanisms through which IgY counteracts pathogen activity have not been determined. But, some mechanisms have been proposed by researchers to express how specific IgY counteracts pathogen activity such as.

i. Aggultination – Tsubokura et al., (1997) have reported that the inhibition of bacterial growth or colonization observed as a result of treatment with IgY due to agglutination, causing a reduction in CFU rather than actual direct effects on individual bacteria.

ii. Adherence-blockade – Most of the in-vitro studies have suggested that inhibition of adhesion is the dominant mechanisms by which specific IgY counteracts pathogen activity (Lee et al., 2002). Particular components exposed on the Gram-negative bacteria surface such as outer membrane protein, lipopolysaccharide, fimbriae (or pili), and flagella, which are crucial factors for bacterial colonization, may be recognized and bound by related polyclonal antibody such as IgY (Sim et al., 2000). This binding may block or impair the functions of growth-related bacterial components and lead bacterial growth inhibition. It is also possible that specific IgY binding to bacteria could alter cellular signaling cascade that could result in decreased toxin production and release.

iii. Opsonization followed by phagocytosis – Nie et al., (2004) determined that IgY improved the phagocytosis of S. aureus by neutrophils. Similarly, Zhen et al., (2008) reported that phagocytic
activity of *E. coli* by either milk macrophages or by polymorphonuclear neutrophil leukocytes increased significantly in the presence of IgY. These results suggest that IgY enhanced phagocytic activity. Structural alterations were observed on the surface of *S. typhimurium* (Lee *et al.*, 2002) and *E. coli* O111 (Zhen *et al.*, 2008b) by binding with specific IgY. These changes could be explained by the variation of the electron cloud and/or electric field on the bacterial surface (Lee *et al.*, 2002), resulting in greater vulnerability of the bacterial cells to phagocytosis.

**iv. Toxin neutralization** - *S. aureus* capsule is a major virulence factor involved in the onset of bovine mastitis. Wang *et al.*, (2011) studied the effectiveness of IgY against encapsulated *S. aureus*. Their results showed that IgY could prevent *S. aureus* internalization by mammary epithelial cells suggesting toxin neutralization activity, rather than direct growth inhibition as the means through which IgY acts to control mastitis (Xu *et al.*, 2011).

### 2.22. Advantages of IgY Technology:

The most important aim of animal welfare is to reduce painful manipulations. IgY technology fulfils this requirement in that chicken Antibodies can be easily sampled by a non-invasive method based on the simple act of collecting eggs. 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 yolk by simple precipitation techniques (Gassmam *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), 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).

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**Fig.6: Advantages of IgY technology (ECVAM Workshop, 1996)**
2.23. Applications of IgY: Chicken polyclonal antibodies were produced against a number of antigens and were applied in many different methods for various purposes; as a research, diagnostic, therapeutic reagents, as a tool for purification or detection of antigens and as a protective agent in passive immunization, where they provide an excellent alternative to or substitution for their mammalian counterparts. IgY was demonstrated to work in practically all tested immunological methods that were traditionally developed for mammalian IgG that is immunofluorescence, immunoenzymes techniques, immunoelectrophoresis and Western blotting, immunohistochemistry and many others. However, one of the most valuable and promising areas of IgY application is its use for passive immunization to treat and prevent human and animal diseases.

Fig.7: General Applications of Chicken IgY

2.24. Egg Yolk Antibodies (IgY) for Passive Immunity: Passive immunity has recently become a more attractive approach because of the emergence of new and drug-resistance microorganisms, diseases that are unresponsive to drug therapy, and individuals with impaired immune systems who are unable to respond to conventional vaccines. Also, passively administered antibodies have the ability to provide rapid and immediate protection; for example, against agents of
bioterrorism (Casadevall et al., 2004). The reduction of antibiotic use in the livestock industry and increasing evidence that resistant organism may pass from animals to humans, resulting in infections that are harder to treat (Yengani and Korver 2010), have led to numerous studies to examine the use of IgY for passive immunity in both human and veterinary medicine.

2.24.1. Passive Immunity versus Active Immunity: Active immunity refers to the process of exposing the individuals to an antigen to generate an adaptive immune response. This response takes days to weeks to develop but may be long lasting. Passive immunity refers to the process of providing preformed antibodies to protect against infection and it provide immediate but short lived protection lasting several weeks to three or four months at most. Passive immunity can be classified as natural or artificial/acquired (Baxter 2007). The immune system of the neonate is relatively immature; therefore maternal antibodies are passed to the offspring in order to confer natural passive immunity until the infant can develop its own immune response. In humans, maternal antibodies (IgG) are transferred from the mother to the fetus during pregnancy and to the neonate through IgA in the breast milk. Other mammals, such as cows, horses, pigs, sheep, and goats, obtain maternal antibodies via colostrum, which are then transported across the intestinal epithelium of the neonate into circulation (Chucri et al., 2010). In contrast, artificial passive immunity involves obtaining antigen-specific antibodies from another source (i.e., an immune individual or animal) and administering it to protect susceptible individuals (Baxter 2007). Because of the transient nature of the protection provided by passively transferred antibodies, repeated or continuous antibody administration is necessary, and large amounts of preformed antigen-specific antibody are required, especially when used orally. Hens’ eggs are an excellent source of large quantities of antibodies that can be used for passive immunization applications.
2.24.2. Applications of IgY in Human and Veterinary Medicine:
Although it is a recent concept in human medicine passive immunization using specific egg yolk 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. In recent decade, there has been increasing interest in the oral administration of specific antibodies to treat antibiotic resistance pathogens, to treat pathogens that do not respond to antibiotics such as viral pathogens and infections of immunocompromised individuals (Casadeval and Scharff, 1995).
Table 10: IgY for passive immunization in Human healthcare, Animal Husbandry and Aquaculture

<table>
<thead>
<tr>
<th>Pathogen/antigen</th>
<th>Target Species</th>
<th>Effects of IgY</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Applications of IgY in Animal Husbandry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Pigs</td>
<td>Protected against infection by K88+, K99+, and 987P+E. coli</td>
<td>Yokoyama <em>et al.</em>, 1992</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>Encapsulated anti-K88+ <em>E. coli</em> IgY enhanced protection and led to improved weight gain</td>
<td>Li <em>et al.</em>, 2009a</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Protected against K99+ <em>E. coli</em> infection in calves</td>
<td>Ikemori <em>et al.</em>, 1992</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Reduced O15:H7 fecal shedding in feedlot steer</td>
<td>DiLorenzo <em>et al.</em>, 2008</td>
</tr>
<tr>
<td></td>
<td>Chicken</td>
<td>Improved intestinal health and immune responses in broilers challenged with O78:K80</td>
<td>Mahdavi <em>et al.</em>, 2010</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Inhibited growth and internalization of O111 and enhanced uptake by macrophages</td>
<td>Zhen <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Chickens</td>
<td>Reduced rate of <em>Salmonella</em>-contaminated eggs in <em>Salmonella Enteritidis</em> (SE)-infected chickens</td>
<td>Gurtler <em>et al.</em>, 2004</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td>Reduced fecal shedding and cecal colonization in SE-infected broilers</td>
<td>Rahimi <em>et al.</em>, 2007</td>
</tr>
<tr>
<td><em>Bovine rotavirus</em> (BRV)</td>
<td>Cattle</td>
<td>Protected neonatal calves from BRV-induced diarrhea</td>
<td>Kuroki <em>et al.</em>, 1994 Vega <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><em>Infectious bursal disease virus</em> (IBDV)</td>
<td>Chickens</td>
<td>Protected chicks from IBDV infections</td>
<td>Yousif <em>et al.</em>, 2006</td>
</tr>
<tr>
<td><em>Porcine epidemic diarrhea virus</em> (PEDV)</td>
<td>Pigs</td>
<td>Protected piglets against PEDV infections</td>
<td>Kweon <em>et al.</em>, 2006</td>
</tr>
<tr>
<td><em>Eimeria spp.</em></td>
<td>Chickens</td>
<td>Protected chicks against avian coccidiosis</td>
<td>Lee <em>et al.</em>, 2009</td>
</tr>
<tr>
<td><em>Canine parvovirus</em> (CPV)</td>
<td>Dogs</td>
<td>Protected dogs against CPV2-induced disease symptoms</td>
<td>Van Nguyen <em>et al.</em>, 2006</td>
</tr>
<tr>
<td><em>Phospholipase A2</em></td>
<td>Chickens</td>
<td>Improved growth and feed efficiency in chickens</td>
<td>Reviewed in Cook 2004,2010</td>
</tr>
</tbody>
</table>

Table Continued...
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Target Species</th>
<th>Effects of IgY</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Applications of IgY in Aquaculture</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Edwardsiella tarda</em></td>
<td>Fish</td>
<td>Prevented <em>E. tarda</em>-induced mortality in eels</td>
<td>Hatta <em>et al.</em>, 1994</td>
</tr>
<tr>
<td><em>Yersinia ruckeri</em></td>
<td>Fish</td>
<td>Reduced mortality and infection rates in rainbow trout</td>
<td>Lee <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><em>Vibrio anguillarum</em></td>
<td>Fish</td>
<td>Protected rainbow trout against <em>Vibriosis</em></td>
<td>Arasteh <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><em>White spot syndrome virus (WSSV)</em></td>
<td>Shrimp</td>
<td>Protected shrimp and crayfish from WSSV infection</td>
<td>Lu <em>et al.</em>, 2008, 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kumaran <em>et al.</em>, 2010</td>
</tr>
<tr>
<td><strong>Applications of IgY in Human Healthcare</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Humans</td>
<td>Reduced binding of O157:H7 in vitro and protected mice from toxin challenge</td>
<td>Wang <em>et al.</em>, 2010</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Humans</td>
<td>Reduced bacterial growth, urease activity and gastric mucosal injury in animal model</td>
<td>Shin <em>et al.</em>, 2002</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Humans</td>
<td>Suppressed <em>H.pylori</em> infection in humans when incorporated into yogurt</td>
<td>Horie <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em> and <em>Streptococcus mutans</em></td>
<td>Humans</td>
<td>Reduced levels of <em>P. gingivalis</em> when applied to the teeth of periodontitis patients</td>
<td>Yokoyama <em>et al.</em>, 2007</td>
</tr>
<tr>
<td></td>
<td>Humans</td>
<td>Reduced levels of <em>S. mutans</em> when used as mouth rinse</td>
<td>Hatta <em>et al.</em>, 1997</td>
</tr>
<tr>
<td></td>
<td>Humans</td>
<td>IgY against <em>S. mutans</em> glucosyltransferase reduced the incidence and severity of dental caries in rats</td>
<td>Kruger <em>et al.</em>, 2004</td>
</tr>
<tr>
<td><em>Tumor necrosis factor-a</em></td>
<td>Humans</td>
<td>Reduced inflammation in experimental colitis in rats</td>
<td>Worledge <em>et al.</em>, 2000</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Humans</td>
<td>Reduced <em>C. albicans</em> colonization in mice</td>
<td>Van Nguyen <em>et al.</em>, 2004</td>
</tr>
</tbody>
</table>

Table continued...
Generation and Characterization of Chicken Egg Yolk Antibodies (IgY) against Salmonella spp. and their Efficacy Studies in Broiler Chicks

### Pathogen Target Species Effects of IgY Reference

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Target Species</th>
<th>Effects of IgY</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza</td>
<td>Humans</td>
<td>Protected mice from lethal H5N1, H5N2 and H1N1 challenge</td>
<td>Nguyen et al., 2010</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>Humans</td>
<td>Blocked activity of neurotoxins A and B in mice</td>
<td>Pauly et al., 2009; Trott et al., 2009</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Humans</td>
<td>Protected monkeys from a lethal dose of <em>S. aureus</em> enterotoxin B</td>
<td>LeClaire et al., 2002</td>
</tr>
<tr>
<td>Venom</td>
<td>Humans</td>
<td>Shown to neutralize pharmacological effects of various venoms</td>
<td>Araujo et al., 2010; Liu et al., 2010; Meenatchisundaram et al., 2008a,b; Paul et al., 2007; de Almeida et al., 2008</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>Humans</td>
<td>Reduced mortality in infected mice</td>
<td>Motoi et al., 2005</td>
</tr>
</tbody>
</table>

(Slightly modified and Adopted from Kovacs-Nolan and Mine, 2012)

It is for this reason that much of the IgY research carried out to use IgY as an alternative to antibiotics and also to mammalian antibodies. The potential applications for using orally administered IgY in the control of enteric and non-enteric infections of either bacterial or viral origins in humans and animals have been studied at length and summarized in Table 10.

### 2.25. Uses of IgY in the control of poultry diseases (Xu et al., 2011)

**a) Salmonellosis:** *Salmonella* infections are thought to be responsible for a variety of acute and chronic diseases of poultry. It has been shown that specific IgY against *Salmonella enteritidis* or *Salmonella typhimurium* inhibits bacterial growth in vitro (Lee et al., 2002). Studies have demonstrated that egg derived anti-*S. enteritidis* IgY antibody had a beneficial effect in reducing the colonization of *Salmonella* in market-aged broilers (Rahimi et al., 2007). Whether or not this translates into improvements in broiler performance has not been determined. The use of whole egg powder (containing antibody) as a feed additive may be an alternative way to reduce the rate of *Salmonella* contamination of eggs. Gurtler et al., (2004) found that in
the experimental group, 13.3% of the eggs were *Salmonella* positive, which was significantly lower than the 29.4% *Salmonella*-positive eggs in the control group. However, it must be pointed out that the majority of the *Salmonella* was found on the shell and there were only modest differences in *Salmonella* numbers in the albumen and yolk. Whether or not this translates into any less health risk for consumers of egg products is not known at this time.

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

**C) Infectious bursal disease:** Infectious bursal disease (IBD) is an acute, highly contagious disease of young chickens caused by IBD virus (Chettle *et al.*, 1989). Early sub-clinical infections which usually result in immunosuppression are the most important form of this disease because of the large economic loss that results from their presence. Chickens immunosuppressed by early IBD infections do not respond well to vaccination and show increased susceptibility to other diseases such as bacterial infection with *E. coli* or viral infections like bronchitis or inclusion body hepatitis (Muhammad *et al.*, 1996). Antibiotic therapy is the most readily-available approach for controlling IBD-induced secondary bacterial infections in affected flocks. It has been shown that specific IgY has a great potential as an alternative to antibiotics for IBD. Muhammad *et al.*, (2001) demonstrated that yolks from hyperimmunized hens can be used to control IBD in commercial laying hens. The IBD infected broilers
(28 days old) treated with the yolk induced 80% recovery while all the control (untreated) birds died.

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

**2.26. Current challenges:** Although beneficial effects of pathogen-specific egg yolk antibodies in animals have been known for about 20 years, results of experimental application of these antibodies to poultry have not always been consistent. Another interesting point is that the quantity of studies concerning possible beneficial effects of IgY in chickens is quite low compared to studies in human and other animal species. There are still many obstacles which make administration of egg yolk antibodies to commercial poultry a difficult goal to achieve. Like any other novel product, it may take a long time to get approval from regulatory authorities in order to use in commercial poultry farms. Finding suitable alternatives to Antibiotic Growth Promoters (AGP) is a high priority for the industry. This lack of immediate availability appears to have diverted attention from IgY to other more readily available alternatives such as organic acids,
prebiotics and probiotics. There is no consensus on what extraction method is most appropriate to use to obtain IgY (Schade et al., 1996). Differences in method can affect yield, purity, stability and efficacy of IgY harvested from the egg yolk (Kitaguchi et al., 2008). Another important consideration is orally-administered antibodies, like any other protein molecule are susceptible to denaturation by the acidic pH of the proventiculus and gizzard and degradation by protease, however a fraction of the administered dose retains some immunological activity against gastrointestinal tract infections (Reilly et al., 2006). It may be possible to develop a protease-resistant oral dosage form of IgY in order to increase the fraction of immunoreactive antibody delivered locally in the gastrointestinal tract. There is an another important factor is cost of IgY use in poultry production than the cost of routine antibiotics, no studies conducted to provide estimation about the production cost of high quality IgY in large scale and its commercial application. The therapeutic or prophylactic application of IgY require the continuous or frequent administration of antibodies, hence the large quantities are required. Cost-effectiveness is a very important determinant to making a shift or implementing a new strategy in the poultry industry (Yegani and Korver, 2010). Hence, further research is needed to overcome the current challenges for therapeutic and prophylactic use of IgY in commercial poultry farms. In particular, there have no studies conducted in India on the commercial applications of IgY in poultry. Therefore, the present study focused on generation and characterization of IgY against Salmonella sp., and their efficacy studies in broiler chicks. The study could form a platform for further research on egg yolk antibodies and its commercial application in India. Poultry are exposed a wide variety of infectious agents in commercial farms. The poultry industry could benefit more from egg yolk antibodies, if they are produced against a variety of common disease-causing microorganisms. If this approach works well, it may help, to some extent, to justify the commercial applications of IgY antibodies as prophylactic and therapeutic agent.