2. Review of Literature


2.1 Mastitis

Milk is one of the most important foods of human beings. It is universally recognized as a complete diet due to its essential components (Javaid et al., 2009). Mastitis is a multietiological complex disease, which is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits et al., 2000). It occurs throughout the world wherever dairy cows are found. Mastitis has been and continues to be recognized as one of the major disease problems concerning the dairy industry. It is also one of the most costly diseases confronting the dairy farmer. Mastitis reduces milk yield and alters milk composition. Amongst cattle diseases, bovine mastitis is a serious problem which affects the basic income of the farmers, depleting their daily sources. Estimating economic losses resulting from mastitis is an extremely difficult task because of the many levels of infection and other factors. The continuing presence of the disease may be attributed to deficient management, improper milking procedures, faulty milking equipment, inadequate housing, and breeding for ever-increasing milk yield. All of these factors are probably involved, although herd investigations often fail to incriminate specific factors. It is important to recognize that mastitis is an infectious disease and that all methods of commercial milk production may provide suitable conditions for spreading mastitis organisms from cow to cow. A considerable body of evidence has accumulated suggesting that several management and environmental factors must interact together to increase exposure of cows to mastitis organisms, reduce the cows natural resistance to disease, or aid organisms in gaining entrance through the teat canal to milk secreting tissues of the udder where they cause infection. The magnitude of these changes in individual cows varies with the severity and duration of the infection and the causative microorganisms. The microorganisms produce toxins that can directly damage milk producing tissue of the mammary gland, and the presence of bacteria initiates inflammation within the mammary tissue in an attempt to eliminate the invading microorganisms. The inflammation contributes to decreased milk production and is primary responsible for the compositional changes observed in milk from infected quarters and cows.
2.2 Effect on Milk Composition

Mastitis causes considerable changes in milk. In general, compositional changes involve an increase in blood components in milk and a decrease in normal milk constituents. Casein, the major milk protein of high nutritional quality, declines and lower quality whey proteins increase which adversely affects the quality of dairy products. Milk always contains a certain amount of somatic cells. Somatic cells are normal constituent of milk and only when they become excessive do they indicate problem. The number of cells reflects the severity of mastitis. In milk obtained from a healthy mammary gland, the Somatic cell count (SCC) is normally lower than $1 \times 10^5$ cells/ml, while in bacterial infection it can increase to above $1 \times 10^6$ cells/ml (Bytyqi et al., 2010). Increased SCC is also associated with reduced suitability of raw milk for manufacturing and processing into products for human consumption. Jones (2006) has reported that with higher SCC, the concentrations of serum albumin and immunoglobulins are increased which reduces heat stability of mastitis milk and pasteurization gives lower grade scores after storage. Also there is a decrease in calcium absorption from blood into milk, resulting impaired coagulation characteristics of mastitis milk. Haenlein et al. (1973) reported a significant decrease in casein content when SCC in milk exceeded 500,000/ml. Potassium, normally the predominant mineral in milk, declines and because most of the calcium in milk is associated with casein, the disruption of casein contributes to lowered calcium in milk. The reduced lactose concentration is one important factor for impaired acidification properties of milk with elevated SCC, after adding starter culture (Schallibaum, 2001).
Table-1 Comparison of values (%) of normal milk with that of mastitis milk having high somatic cell count

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Normal milk</th>
<th>Mastitis milk with high SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Total Protein</td>
<td>3.61</td>
<td>3.56</td>
</tr>
<tr>
<td>Total Casein</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Whey Protein</td>
<td>0.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Serum Albumin</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Lactoferin</td>
<td>0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>0.1</td>
<td>0.60</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.057</td>
<td>0.105</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.091</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Source: Jones (2006)

2.3 Etiology

Mastitis is an inflammation of the milk secreting tissue of the udder caused by bacterial infections. Today's mastitis is considered to be a multifactorial disease. Over 200 microbial species, sub species and serotypes have been isolated from bovine mammary gland (Mallikarjunaswamy and Krishnamurthy, 1997) and identified as causative agents of mastitis. Apart of different species of bacteria, several other groups of micro-organisms such as virus, fungi, yeast, algae and chlamydia can cause mastitis in cattle and buffaloes.

Mastitis is caused by many Gram positive and Gram negative bacteria. In India, *Staphylococcus*, *Streptococcus* and *E.coli* generally cause 90-95% of all infections of mammary gland (mastitis). The goal of every dairy farmer should be to minimize the number of organisms permitted to come into contact with the teats. Fortunately, the vast majority of mastitis cases are caused by a relatively small number of microorganisms that can be grouped into three categories: (1) Contagious; (2) Environmental and (3) other.
A) **Contagious:** The important organisms of this group are *Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium bovis* and *Mycoplasma* species. These spread from infected to clean udders during the milking process through contaminated milker’s hands and possibly by flies.

B) **Environmental:** The important organisms of this group are *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Serratia* species, *Citrobacter* species, *Enterobacter aerogenes, Streptococcus uberis, Streptococcus bovis* and *Streptococcus dysgalactiae*. Transmission of these pathogens may occur during milking but primarily between milkings.

C) **Other organisms:** The vast majority of organisms in this group rarely cause clinical mastitis and are not of serious economic importance to the dairy industry, though they do infrequently cause serious problems in dairy herds that do not practice good management including Coagulase-negative *Staphylococci* (CNS), *Serratia* species, *Pseudomonas aeruginosa, Nocardia asteroides, Prototheca* species, *Candida* species (yeasts), *Arcanobacterium pyogenes* (*Corynebacterium pyogenes*).

### 2.4 Types of Mastitis

It is important to be able to recognize the different types of mastitis in order to decide what preventive measures or treatments to use. Following are the different types of mastitis and there characteristics.

A) **Contagious:** Mastitis caused by bacteria such as *Staphylococcus aureus* and *Streptococcus agalactiae*, of which the main source are other infected cows.

B) **Environmental:** Mastitis caused by bacteria such as coliforms of which main source is contaminated environment.

C) **Acute Clinical:** Inflammation of the teat, fever above 39°C, weak and dejected animal, lack of appetite, drastic drop in milk yield.
**D)** **Hyperacute Clinical:** Swollen, red, painful quarter. Milk passes with difficulty. Fever over 41°C, loss of appetite, shivering, weight loss and lactation often stops.

**E)** **Subacute Clinical:** No apparent change in udder, presence of flaky particles in milk, especially in initial ejection. Cow appears healthy.

**F)** **Subclinical:** No symptoms. Milk appears normal. Only change is detection of pathogenic agent in analysis and increased somatic cell count.

**G)** **Chronic:** Repeated but mild clinical attacks, generally without fever. Lumpy milk, quarters sometimes swollen, quarter may become hard. Antibiotic treatments often do not work.

**H)** **Gangrenous:** Affected quarter is blue and cold to touch, progressive discoloration from the tip to the top, necrotic parts drop off and cow often dies.

### 2.5 Coliform Mastitis

The term coliform mastitis frequently is used to identify mammary gland disease caused by all Gram-negative bacteria. Coliform bacteria are normal inhabitants of soil, digestive tract and manure. They accumulate and multiply in contaminated bedding. Research has shown that coliform numbers of 1,000,000 or more per gram of bedding increase the likelihood of an udder infection and clinical mastitis. Gram-negative bacteria are the etiological agents most often isolated from acute clinical cases of mastitis. The point sources of coliform bacteria that cause infections include bedding materials, soil, manure and other organic matter in the environment of cows. Genera classified as coliforms are *Escherichia*, *Klebsiella*, and *Enterobacter*. Other Gram-negative bacteria frequently isolated from intramammary infections include species of *Serratia*, *Pseudomonas*, and *Proteus*. Transfer of Gram-negative bacteria from the mammary glands of infected cows to uninfected cows appears minimal compared with the constant environmental exposure. Coliform bacteria occupy many habitats in the cow’s environment. *Escherichia coli* are normal inhabitants of the gastrointestinal tract of warm blooded animals. Both *Klebsiella* spp. and *Enterobacter* spp. populate soil, grains, water, and intestinal tracts of animals.
Coliform bacteria are among the etiological agents commonly responsible for infectious respiratory and urogenital diseases in dairy cows. However, the spread of Gram-negative bacteria from other regions of the body to the mammary gland via the vascular or lymphatic systems appears minimal. Intramammary infections caused by Gram-negative bacteria typically result from the bacteria traversing the teat canal and multiplying in the gland. Although the mammary gland is not considered a natural habitat for coliform bacteria, many strains are capable of surviving and multiplying in the mammary gland.

According to Kennedy and Miller (1993), mastitis is expressed by tissue injury caused by tissue invasive or toxigenic organisms, which become dominant due to upset of balance in microbial population. The recent scientific literature on mastitis is so vast that the people have concentrated on individual species of major mastitides and its various aspects of treatment and control (Kirk et al., 1988; Saran, 1995). *Escherichia coli* is among the most common infectious agents isolated from severe mastitis cases in modern dairy farms (Hogan et al., 1989; Bradley et al., 2007). Factors that are cow-dependent, like the speed of the inflammatory response, lactation stage and age of the cow, are thought to determine the severity of *E.coli* mastitis (Burvenich et al., 2003). Teat end hyperkeratosis and a dirty udder significantly increase the risk of clinical *E.coli* mastitis (Breen et al., 2009). Coliform bacteria are responsible for a great number of acute clinical cases in dairy cows. Once coliforms enter the mammary gland, they often multiply rapidly or may remain dormant for several days. As they multiply, coliforms produce endotoxins which are subsequently released when the bacteria are destroyed by leucocytes. Once released from the bacteria, the toxins are absorbed into the bloodstream. A cow affected by the toxins will show signs of high fever, depressed appetite, rapid weight loss, abnormal milk and decreased production within a few hours. Sometimes enough endotoxin is released which result in serious illness and death of cows.
Review of Literature

Prevention of Clinical Coliform (Escherichia coli) Mastitis in Dairy Cows by Specific Chicken Egg Yolk Immunoglobulin (IgY) in Coimbatore District

Plate 1a – Cow infected with Mastitis. Mastitis is persistent and potentially fatal mammary gland infection, leading to high somatic cell counts and loss of milk production.

Plate 1b - Inflammation and hardening of udder with discoloration of milk
In about 10 percent of clinical coliform mastitis cases, cows die within one to two days after the infection becomes apparent, in spite of aggressive veterinary care. Diagnoses of intramammary infections caused by Gram negative bacteria offers a number of unique challenges compared with other mastitis pathogens (Hogan et al., 1999). Colony forming units in milk often are less than 100 cfu/ml for Gram negative bacteria isolated in the later phase of clinical disease or from subclinical glands. The two main preventive measures of intra mammary infection are to minimize the challenge from the contaminated environment and to maximize the cow’s own defense (Bradley and Green, 2004). One of the primary sources of bacterial contamination is bedding contaminated with manure. Management practices that reduce the number of bacteria in the environment of the cow generally decrease the occurrence of clinical mastitis caused by Gram negative bacteria (Hogan and Smith, 2003).

E.coli mastitis is an important case of mastitis on modern dairy farms. The pattern of disease seems to have changed from principally acute toxic mastitis to sub acute clinical mastitis. The case of this change is unclear. The possibility that this is related to the changes in bacteria causing the disease seems unlikely as there is no evidence that virulence factors or bacterial phylogeny are associated with disease severity. Coliform mastitis seems to be the result of opportunistic infection with no evidence of udder- adapted strains playing an important role even in persisting infections. This highlights once again that prevention of infection from the environment remains key to the control of E.coli mastitis (Richard Laven, 2013).

2.6 Economic Implication of Mastitis in Dairy Animals

Mastitis continues to be a significant economic loss to the dairy industry internationally. Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses (Sharma et al., 2007). Mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Bhikane and Kawitkar, 2000). Economic losses are estimated to be approximately $200 per cow per year in the U.S. and these losses are due to
reduced production, increased replacement costs, discarded milk, drug costs, veterinary fees, and labour costs. Apart from its economic importance it also carries public health significance.

Kaneene and Hurd (1990) reported the average cost of mastitis EUR 28 per cow per year and the average cost of mastitis prevention was EUR 3.56 per cow per year, varying from EUR 0 to EUR 22 in Michigan. Hillerton et al. (1992) calculated the cost of summer mastitis in 95 herds in England. They found that only summer mastitis, on an average, costs EUR 279 per case per year. A loss was reported of EUR 9.03 billion per year to the UK industry due to summer mastitis.

The annual loss per cow from mastitis in the United States in 1976 were estimated to be $117.35 and losses of milk yields caused by mastitis were 386kg/cow per year and losses of discarded milk 62kg/cow per year (Blosser, 1979). While these losses increased upto $185 to $200 per cow per year (Costello, 2004). In 1976 losses from mastitis were 1.294 billion in U.S. and increased upto $2 billion in the year of 2009 (Viguier et al., 2009). In Canada, it is estimated that mastitis costs dairy producer $750/cow/year in terms of lower milk production, cost of medicine, treatment time and premature culling. Moreover, 70 to 80% of that loss is due to subclinical mastitis, which is non-symptomatic (Kirk and Bartlett, 1988; Natzke, 1981).

The first comprehensive report on mastitis caused losses in India published in 1962 indicated annual losses of Rs. 52.9 crores (Dandha and Sethi, 1962). However tremendous thrust on cross breeding programme and launching of operation flood in later years resulted in tremendous increase in high yielding bovine population, leading to many fold increase in economic loss. This is evidenced from a recent report where in annual economic losses incurred by dairy industry in India on account of udder infections have been estimated about Rs.6053.21 crores. Out of this, loss of Rs. 4365.32 crore (70 % - 80 % loss) has been attributed to sub clinical version of udder infections. In addition to heavy losses in milk quality and quantity, it also causes irreversible damage to the udder tissue and less occasional fatalities (Dua, 2001).
In both clinical and subclinical mastitis there is a substantial loss in milk production. Janzen (1970) cited losses of milk per quarter per day in mastitic cows of 0.34 to 2.66 kg (9.0 to 43.3%). A study by Wilson et al. (2004) reported that mastitis often hits the cows with the highest production potential, which expands the loss due to mastitis. According to the study, the estimated loss following clinical mastitis was almost 700 kg for cows in first lactation and 1,200 kg for cows in second or higher lactation.

As per 2006 estimates referred in ICAR’s National Agricultural Innovation Project, the estimated annual loss due to mastitis alone is nearly Rs. 16,702 millions. Naveen Kumar et al. (2010) reported that the economic loss per annum due to clinical mastitis in Kolar district, Karnataka was estimated to be 1.48 crores.

It is important to emphasize that the modern dairy cow is completely dependent upon man, who also is the most important component of management. Since cows cannot communicate directly with us concerning deficiencies in management, environment, and hygiene, there is no substitute for keen observation by owners and herdsmen to try and maintain management at a high level. Indeed, the level of management is more important in mastitis control than the specific management factors being followed. Furthermore, a positive attitude toward mastitis control is essential for success because there is no magic potion, although the perseverance and strict attention to detail will eventually yield excellent result.

2.7 Status of Mastitis in Dairy Animals in India

In India, the teat dipping as a preventive measure is not regularly practiced by dairy farmers; hence, it is essential to educate the farmers regarding the risk factors of mastitis and also about teat dipping (Kavitha et al., 2009). Surveys of the prevalence of mastitis in most countries, irrespective of the cause, show a comparable figure of 50% among dairy cows and a quarter infection rates of 25% (Radostits et al., 2000). Subclinical mastitis is believed to be more prevalent than clinical mastitis in most countries. Sharma et al. (2006) had been reported 36.69% and 16.78% prevalence of mastitis at cow and quarter level, respectively in subclinical mastitis affected cross bred cows (Sahiwal and Jersey) by cultural examination from Haryana. A study from Rajasthan showed
60.25% prevalence in cows and 39.00% in quarters by cultural examination, and highest prevalence was found in 6th lactation on quarter basis and 3rd lactation on animal basis (Chahar et al., 2005). De and Mukharjee (2009) have been reported the overall prevalence of clinical mastitis and subclinical mastitis were 15.18% and 42.93% respectively during the month of July and August in Uttar Pradesh. Sharma (2003) had been reported prevalence of subclinical mastitis in cows and buffaloes 78.54% and 68.60% respectively, by MCMT in Chattisgarh State. Sharma et al. (2009) had been reported prevalence of subclinical and clinical mastitis 42.18% and 10.93% respectively in dairy cows in Jammu. A study in Jammu by Sudhan et al. (2005) suggests that *Staphylococcus aureus* (56.89%) is major pathogen followed by *Micrococcus* spp. (15.51%), *Bacillus cereus* (12.06%), *Staphylococcus epidermidis* (8.62%), *Klebsiella* spp. (3.44%), *Escherichia coli* (1.72%), and *Corynebacterium* spp. (1.72%). Sharma et al. (2007) also isolated *Staphylococcus aureus* and *E. coli* from acute clinical mastitis in buffalo. Muhammed Mubarak et al. (2012) worked on prevalence of bovine mastitis on dairy cows in and around Coimbatore district and reported that out of 250 lactating cows tested, prevalence of mastitis at cow level was 66.0% (165/250), out of which 26.06 % (43/165) and 75.30 % (122/165) were clinical and subclinical, respectively.

### 2.8. Factors Affecting the Susceptibility to Mastitis

The large number of predisposing factors that contribute to the emergence of mastitis in dairy cattle may be physiological, genetic, pathological or environmental (Sordillo, 2005).

#### 2.8.1. Age of the Cow

It has been demonstrated that occurrence of mastitis in infected quarters increases with age in cows (Harmon, 1994; Sharma and Maiti, 2010). This may be due to an increased cellular response to intramammary infection or due to permanent udder tissue damage resulting from the primary infection (Dullin et al., 1988). However, the study conducted using 4133 cattle including both cross-bred and non-descriptive breeds revealed the highest risk of occurrence of mastitis to be between the age of 4-6 years, followed by the age group between 2-4 years, with the least occurrence noted between 6-8 years of age (Mahajan et al., 2011).
2.8.2. Inherited features of Bovine

Various genetic traits may also have a considerable impact upon the susceptibility of the animal to mastitis. These genetic traits include the natural resistance, teat shape and conformation, positioning of udders, relative distance between teats, milk yield and fat content of milk. High milk yielders with higher than average fat content are reported to be more susceptible to mastitis (Lactation Resource Library, 2009).

2.8.3. Stage of Lactation

The incidence of mastitis is reported to be higher immediately after parturition, early lactation and during the dry period, especially the first 2-3 weeks (Corbett, 2009; Fadlelmula et al., 2009). It will be probably due to increased oxidative stress and reduced antioxidant defence mechanisms during early lactation (Sharma et al., 2011).

2.8.4. Mammary regression

There are significant functional changes in the udder during the early and late lactation and dry period, which affect the cow’s susceptibility to infections. Lactating cows under stress show premature mammary regression. Such a condition compromises udder’s natural defence mechanisms leading to invasion of the teat canals by potential pathogens (Capuco et al., 2003).

2.8.5. Milking Machine

Extraneous factors such as the milking habits of farmers and faulty milking machines favour the pathogens to gain access to mammary gland and proliferate, potentially leading to mastitis (Mein et al., 2004). Proper installation as well as the correct maintenance of milking machines is important to avoid an inadequate vacuum level, teat and tissue damage and incomplete milking (International Dairy Federation, 1987). There is a report of increased risk of both contagious and environmental mastitis causing pathogen due to machine induced changes, which widen the orifice of the teat canal in cows (Mein et al., 2001).
2.8.6. Nutrition

The quality and plan of nutrition appears to be an important factor that influences clinical manifestation of mastitis in heifers and cows (Heinrichs et al., 2009) although no relationship between the incidence of mastitis and either high energy or high protein feed in cows has been reported (Rodenburg, 2012). Vitamin E is one of the important supplements in dairy feed to boost the immune response of cows (Spears and Weiss, 2008). The neutrophils of selenium fed cows are more effective at killing mastitis causing microorganisms than those not supplemented with selenium (Erskine et al., 1989; Grasso et al., 1990). Zinc and copper are also important nutritional elements that contribute mammary gland health by promoting cellular repair, wound healing and reduction in SCC (Bruno, 2010). Feed supplemented with copper and fed to heifers reduces the severity of subclinical mastitis as well as clinical mastitis induced by Escherichia coli (Upadhayay et al., 2008).

2.8.7. Weather and Climate

The incidence of mastitis is greatly influenced by the weather conditions and prevailing climatic conditions. Heat, humidity, cold and draught are the important predisposing factors (Dhakal et al., 2007; Reneau, 2012). A higher incidence of mastitis has been reported to occur particularly during summer rainy months (Sentitula and Kumar, 2012). As heat and humidity increases, so does the bacterial multiplication as well as the load of pathogens in the environment (Godden et al., 2003).

2.9 Diagnosis

Diagnosis of clinical mastitis is based on the appearance of abnormally appearing milk. Milk may be off color, watery, bloody or have the appearance of serum. Abnormal milk may also contain varying amounts of pus and clots. The amount of swelling, severity of pain and the overall appearance of the cow will indicate the severity of infection and serve as a guide for the course of treatment. Diagnosis of subclinical infection is more problematic since the milk appears normal but usually has an elevated somatic cell count. Diagnosis of subclinical mastitis can be made in a variety of ways including
direct measurement of the somatic cell count (SCC) level or indirectly by performing a California Mastitis Test (CMT) on suspected quarters. Milk culture of suspected quarters or cows will identify the presence of mastitis pathogens but will not provide a measure of the degree of inflammation associated with the infection. For diagnosing mastitis physical examination of the udder should be done to observe any deviation normal shape, size, color and consistency of udder. Signs of inflammation like heat, swelling, pain, redness and loss of function should be clinically assessed. Diagnostic tests of milk include Strip Cup Test, Bromothymol Blue Test, Bromocresol Purple Test, Chloride Test, Catalase Test, Hotist Test, California Mastitis Test, White Side Test, Modified California Mastitis Test, CAMP Test, Draminski Mastitis Detector and Electrical Conductivity Test. Direct tests are cultural examination, biochemical tests, animal inoculation test, serological tests etc. Molecular diagnostic technologies also have been used as a routine tool in diagnosing mastitis pathogens (Gurjar et al., 2012).

2.10 Prevention and Therapy of Mastitis

Prevention is the key in mastitis control. A control program should emphasize factors that reduce the rate of new infections. New infections are controlled by adopting measures like proper milking procedures, improved milking hygiene and housing management (Arnold and Jeffrey, 2011). The prevention of mastitis can be achieved by:

2.10.1. Proper milking hygiene

Bacteria transmit to the uninfected from the contaminated hands of the milker. Thus the milker’s hands should be washed thoroughly with disinfected soaps before milking and clinically infected cows should be milked last. Teats should be cleaned and dried before milking. Poor hygienic conditions can lead to *E.coli* mastitis as the udder is infected through teat canal (Sumathi et al., 2008).
2.10.2. Milking machine

Should function and operate properly. Vacuum level in the milking unit should be between 275 and 300 mm of mercury with little fluctuation. The vacuum regulator should be kept clean and checked regularly.

2.10.3. Dipping teats after milking

Teat dipping does not reduce existing infection. However, the rate of new infection can be reduced by up to 50% when suitable disinfectant is used to immerse or spray the teats.

2.10.4. Dry treatments

Incidence of mastitis during the dry period can be considerably reduced by effective use of antibiotic infused in each quarter of the udder at the last milking of lactation. Dry cow therapy is the best way to cure chronic and subclinical mastitis that are difficult to treat successfully during lactation.

2.10.5. Culling of chronically infected cows

This is an effective method because in most herds only 6-8% of all cows account for 40-50% of all clinical mastitis. Cull animals that are severely or repeatedly affected by mastitis. Cows with injured teats that do not heal should be put at the top of the list of animals to cull. Cows that maintain a high cell count during all lactations should also be culled.

2.10.6. Nutrition

Deficiencies of selenium and vitamin E in the diet have been associated with an increased rate of new mammary infections. Proper nutrition will reduce the risk of environmental mastitis. Adequate levels of Vitamin E and selenium reduce the incidence of environmental mastitis.
2.10.7. Antibiotic therapy

Antibiotics ranging from narrow to broad spectrum have been used extensively over the past 40 years in the control of bovine mastitis (Barkema et al., 2006). However, because of the emerging antibiotic resistance believed to be probably due to their overuse (Gao et al., 2012) and the induction of prolonged persistent antibiotic resistance in biofilms by many mastitis causing pathogens, as demonstrated recently for S.aureus isolated from cases of bovine mastitis (Babra et al., 2012), effectiveness of antibiotic therapy has been compromised. As such the control of bovine mastitis has become one of the most challenging problems on dairy farms today. Currently available antibiotics have minimal effect on shortening the duration of intramammary infections caused by coliform bacteria. Till date broad spectrum antibiotics are injected to reduce financial loss. It leads to serious side effects. The number of antimicrobials suitable for systematic treatment of coliform mastitis is limited and very few antimicrobial products have been approved specifically for this indication and even fewer have demonstrated favourable pharmacokinetic and pharmacodynamic properties (Constable et al., 2008). Broad spectrum antimicrobials such as fluoroquinolones, cefquinome, ceftiofur and oxytetracycline have been used or recommended for the treatment of E.coli mastitis (Huxley, 2004; Erskine et al., 2002). Broad spectrum antimicrobials, commonly used for mastitis treatment, are of major therapeutic importance also in human medicine, and their broad use in food producing animals has stimulated public health concerns (Collignon et al., 2009).

Non antimicrobial approaches for treating E.coli mastitis have been studied as alternatives to antimicrobials. Non-steroidal anti-inflammatory drugs (NSAID), frequent milking and fluid therapy have been commonly recommended for supportive treatment of coliform mastitis (Radostits et al., 2007).

Application of hygienic measures during milk collection, using milking machines, lactation and dry cow therapy, teat sealers, dietary supplements and culling are likely to reduce but not control the incidence of both clinical and subclinical mastitis. The effects of mastitis on dairy cattle health and milk production highlight an urgent need to develop effective strategy of prevention and control (Tiwari et al., 2013).
2.11 Chicken Antibodies as an alternative source to Mammalian Antibodies

Antibodies presently available for research, diagnostic and therapeutic are mostly mammalian monoclonal or polyclonal antibodies. Traditionally, commercially available polyclonal antibodies have been produced in mammals such as mice, rats, rabbits, sheep, goats, and horses, and are generally obtained from sera after immunization of these animals (Schade et al., 1991). The antibodies obtained from blood of these animals were collected either by repeated bleeding or heart puncture resulting in death of the animal. Mammalian antibodies, however, could be substituted with immunoglobulins obtained from avian eggs. Evidence is accumulating that egg antibodies possess properties comparable or in some regards even better than those of mammalian ones (Carlander et al., 1999). Disadvantages of the available techniques and concern for animal rights enhance the interest in developing alternative methods for the production of antibodies.

Hens’ eggs have long been recognized as sources of nutrients, including large quantities of egg yolk antibodies. Immunoglobulin Y (IgY) is the functional equivalent of immunoglobulin G (IgG) in mammals and is transferred to the yolk to passively protect the developing chick. Three classes of antibody are found in the chicken: IgY, IgA, and IgM (Leslie & Martin 1973). During egg formation, IgY in the serum is selectively transferred to the yolk via a receptor on the surface of the yolk membrane specific for IgY translocation (Morrison et al., 2002; Tesar et al., 2008), whereas IgA and IgM are deposited into the egg white (Rose et al., 1974).

Because of differences in the immunoreactivities of IgY and IgG, egg yolk antibodies have been used in many diagnostic and biomarker discovery applications. However, much research has focused on the use of IgY for passive immunization application. Passive immunization has recently become an even more attractive approach because of the emergence of new and drug-resistant 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) (Figure 1).
Fig 1 - (a) Active immunity involves immunizing, or vaccinating, an individual with antigen to generate an adaptive immune response targeting the pathogen of interest. (b) In passive immunization, antibodies are isolated from another source (e.g., the yolks of immunized hens) and administered to susceptible individuals to provide pathogen-specific immunity (Hatta et al., 2008).
2.12 Structure and Characteristics of IgY

The predominant class of immunoglobulin in chicken is called IgY, which is transferred from serum to the yolk for protection of the embryo against the infections (Larsson and Sjoquist, 1990). Chickens store high contents of IgY-antibodies in the yolk and are considered to be efficient antibody producers (Almeida et al., 2008). In 1969 Leslie and Clem showed experimental data proving profound differences in their structure and proposed the name IgY. Now IgY is recognized as a typical low-molecular-weight serum antibody of birds, reptiles, amphibia and lungfish, and as an evolutionary ancestor of IgG and IgE antibodies that are unique to mammals only. Among all birds, chicken IgY is most frequently studied, best described and characterised. General structure of IgY molecule is the same as of IgG with 2 heavy (Hv) chains with a molecular mass of 67–70 kDa each and two light (L) chains with the molecular mass of 25 kDa each. The major difference is the number of constant regions (C) in H chains: IgG has 3 C regions (Cv1 – Cv3), while IgY has 4 C regions (Cv1 – Cv4). One additional C region with two corresponding carbohydrate chains has a logical consequence in a greater molecular mass of IgY compared to IgG i.e. 180 and 150 kDa, respectively. IgY is much less flexible than IgG due to the absence of the hinge between Cv1 and Cv2, which is a unique mammalian feature. There are some regions in IgY (near the boundaries of Cv1–Cv2 and Cv2–Cv3) containing proline and glycine residues enabling only limited flexibility. IgY has isoelectric point 5.7–7.6 and is more hydrophobic than IgG. Regarding the relatively high core body temperature of chickens, which is 41 °C, it is not surprising, that half-life time of IgY is in months and that they retain their activity after 6 months at room temperature or for one month at 37°C.

Fig 2 - Structure of IgY (Losonczy, S. and J.Batke. 1997)
IgY is fairly heat stable and most antibody activity remain after 15 min at 70°C. Incubation of IgY at pH above 4 is well tolerated, but at pH 2 and 37°C the activity is rapidly decreased. The rapid activity loss is probably due to conformational changes, as the polypeptide is not broken down as observed by SDS-PAGE. The immunological activity of IgY is not affected by pasteurization at 60°C for 3.5 min. Addition of high concentrations of sucrose stabilizes IgY regarding heat denaturation, acid environment as well as high pressure. IgY fractions have been stored in 0.9% NaCl, 0.02% NaN₃ at +4°C for over 10 years without any significant loss of antibody titer. IgY, like IgG, has been found to be relatively resistant to trypsin and chymotrypsin digestion, but sensitive to pepsin digestion (Shimizu et al., 1988).

| Table 2 - Comparison of the characteristics of mammalian IgG and avian IgY (Schade et al., 1991). |
|--------------------------------------------------|--------------------------------------------------|
| **Mammalian IgG** | **Avian IgY** |
| Antibody sampling | Invasive | Non-invasive |
| Antibody amount | 200mg IgG per bleed (40 ml blood) | 50-100mg IgY per egg (5-7 eggs per week) |
| Amount of antibody per month | 200 mg | ~ 1500 mg |
| Amount of specific antibody | ~ 5% | 2-10% |
| Protein-A/G binding | Yes | No |
| Interference with mammalian IgG | Yes | No |
| Interference with rheumatoid factor | Yes | No |
| Activation of mammalian complement | Yes | No |
2.13 Immunization

The production of large amounts of IgY in a cost-effective manner is key to its successful use for passive immunization. Different aspects of hen immunization have been studied in order to improve IgY production and yolk deposition, including immunization route, vaccine adjuvant, and type of antigen (Levesque et al., 2007). The most common injection route is the intramuscular route, and Chang et al. (1999) demonstrated that intramuscular immunization resulted in higher levels of specific IgY when compared to antigen injected subcutaneously. Usually 10–100µg of protein antigen in a final volume of 1ml is applied intramuscularly in the breast muscle at two or three injection sites of 7 to 8 week-old chicken. Injection of the antigen by intramuscular route frequently results in higher antibody levels by day 28th after immunization (Wooley & Landon, 1995). Chickens immunized by the intramuscular route continue producing specific antibodies during more than 200 days or can be used for the entire laying period depending basically on the antibody titres induced (Horton et al., 1985; Schade et al., 1996). The presence of yolk antibodies should be checked two weeks after the second immunization. When the antibody titer decreases booster immunizations can be given during the whole laying period. A laying hen produces five to six eggs per week. Average volume of egg yolk (15ml) contains 50–100 mg of IgY, of which 2 to 10% are specific antibodies.

2.14 Production and purification of immunoglobulin Y

Chicken eggs present an ideal alternative antibody source to mammals, as the IgY in the chickens blood is transported to the egg and accumulates in the egg yolk in large quantities. Egg yolk contains a considerable amount of IgY, around 100-150 mg per egg (Rose et al., 1974). Although the amount of IgY deposited into the yolk varies depending on several factors, including the age, breed of chicken, and antigen used, IgY yields have been reported to range from 60 to 150 mg IgY per egg (Cook & Trott 2010, Pauly et al., 2009). Given that a typical hen can lay approximately 325 eggs per year, this can result in a potential yield of around 20–40 g of IgY per year (Pauly et al., 2009), of which 2% to 10% is antigen-specific (Schade et al., 1991, Tini et al., 2002).
One of the major challenges in IgY purification is separating the water soluble IgY from the yolk lipoproteins (Hatta et al., 2008), and a number of methods have been reported that result in different yields and purities. This typically involves isolating the IgY-containing water soluble fraction, followed by additional purification steps. Dilution of the yolk with water, which results in the aggregation of yolk lipoproteins at low ionic strength (Jensenius et al., 1981), followed by centrifugation or ultrafiltration, has been reported (Akita & Nakai, 1992; Kim & Nakai, 1996, 1998). Likewise, freezing and thawing of diluted yolk, producing lipid aggregates that are large enough to be removed by conventional low speed centrifugation, have also been used (Jensenius & Koch, 1993), resulting in a purity of approximately 70% (Deignan et al., 2000). For dilution methods, pH and extent of dilution are very important for optimal IgY recovery, and Nakai et al. (1994) found that the best results were obtained using a six-fold water dilution, at pH 5.0.

Other methods of removing lipoproteins prior to IgY purification include organic solvent delipidation (Horikoshi et al., 1993; Kwan et al., 1991; Polson, 1990) and use of lipoprotein coagulating agents, such as polyethylene glycol (Akita & Nakai, 1993a; Polson et al., 1980; Svendsen et al., 1995) or dextran sulfate (Jensenius et al., 1981); however, application of these methods for large-scale production of IgY for passive immunization are limited by problems related to safety as well as cost constraints (Hatta et al., 2008). As an alternative, natural polysaccharides, including sodium alginate (Hatta et al., 1988), xanthan gum (Akita & Nakai, 1993a), λ-carrageenan (Hatta et al., 1990), and pectin have been found to be just as effective, precipitating more than 90% of lipoproteins from yolk (Chang et al., 2000).

A particularly efficient method consists of two successive precipitations in PEG, using 3.5% PEG to remove fatty substances, and then 12% PEG to precipitate the IgY. An improvement of this method, incorporates an emulsification step, adding one volume of chloroform rather than using 3.5% PEG (Polson et al., 1980 and Polson, 1985). It is generally assumed that about 100mg of IgY can be recovered per egg yolk.
2.15. Applications of IgY Administration

2.15.1. Application of IgY in the control of livestock diseases

A) Bovine mastitis

Bovine mastitis is a costly disease for the dairy industry. Numerous pathogens can cause mastitis and these can be classified into contagious pathogens (primary Staphylococcus aureus and Streptococcus agalactiae) or environmental pathogens (primary E. coli; Rifon et al., 2001). The specific IgY produced by hens immunized with S. aureus and/or E. coli was effective in controlling experimental and clinical mastitis (Zhen et al., 2008a,b; 2009) they also reported that the efficacy of specific IgY to reduce clinical and experimental mastitis caused by S. aureus was demonstrated by improving milk quality through a decrease in somatic cell and bacterial counts in milk. The cure rates resulting from the use of IgY for clinical and experimental mastitis were dramatically higher than for untreated animals and these studies indicate that specific IgY has considerable potential as a therapeutic treatment for mastitis in dairy cow.

B) Diarrhea in piglets

Enterotoxigenic E. coli (ETEC) is by far the most common cause of enteric colibacillosis encountered in neonatal and post-weaned pigs (Yokoyama et al., 1992). The strains of E. coli associated with intestinal colonization which cause severe diarrhea are the K88, K99 and 987P fimbrial adhesins. Among the ETEC, those expressing the K88+ fimbrial antigen are the most prevalent forms causing E. coli infection world-wide (Rapacz and Hasler-Rapacz, 1986). It has been estimated that K88+ ETEC are responsible for more than half of the piglet mortality which occurs each year (Waters and Sellwood, 1982), causing significant economic loss for the pig industry. Yokoyama et al. (1992) showed that orally administered IgY generated against E. coli K88, K99, or 987P fimbriae was protective against infection from each of the three homologous strains of E. coli in a dose-dependent manner. A group of researchers at the University of Manitoba (Winnipeg, Canada) have carried out some excellent studies on the passive protective effect of IgY against ETEC K88 fimbriae in the control of neonatal and early-weaned piglets in vitro and in vivo (Jin et al., 1998; Marquardt et al., 1999)
C) Diarrhea in calves

Neonatal calf diarrhea caused by bovine rotavirus (BRV) is a common disease and causes high mortality in cattle (Lee et al., 1995). Anti-BRV IgY has been shown to successfully provide protection against BRV infection in calves (Kuroki et al., 1994). Bovine corona virus (BCV) is an important enteric pathogen that is responsible for both neonatal calf diarrhea and acute diarrhea in adult cattle. BCV may be more severe than BRV as it replicates in the epithelium of both the small and large intestine whereas BRV infects only the small intestine. Ikemori et al. (1997) examined the efficacy of specific IgY antibodies and cow colostrum antibodies to BCV-induced diarrhea in calves. They found that control calves which received no antibodies developed severe diarrhea resulting in all calves dying within 6 days of BCV challenge whereas calves treated with milk containing egg yolk or colostrum had positive weight gains and none of these calves died. These results do indicate that orally administered egg yolk or colostral antibodies have the potential to passively protect calves against BCV infection and the effect of IgY was higher than that of colostrum alone indicating that the passive immunization strategy with IgY provided a more efficacious alternative to existing treatments for BCV.

2.15.2. Applications of IgY in the control of poultry diseases

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). Diraviyam et al. (2011a) worked on in vitro studies of chicken egg yolk antibodies generated against Salmonella pullorum and reported that the generated IgY was specific against S. pullorum whole cell antigen and it could effectively bind with that. The raised antibodies could be used for the passive immunotherapy to protect the young chicks from horizontal transmission of Pullorum disease by improving the immunological strength against infectious disease.
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 faeces was 80–95% lower.

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). The 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.

2.15.3. Applications of IgY in the control of aquatic diseases

A) Shrimp white spot syndrome virus

White spot syndrome virus (WSSV) is a virulent pathogen causing high mortality and significant economic loss in cultured shrimp operations worldwide (Wongteerasupaya et al., 1995). WSSV can be neutralized by chicken IgY produced against a truncated fusion protein of VP28 and VP19 (Kim et al., 2004) and therefore, passive immunization with IgY against WSSV has potential for immunotherapeutic application to prevent WSSV infection in shrimp.

B) *Yersinia ruckeri*

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

2.16 Development of Chicken Egg Yolk Antibodies

According to Polson et al. (1980) high levels of antibody activity in egg yolk was maintained for several months by periodic immunization. Furthermore, vaccination of small animals such as chickens can be performed easily. Chicken antibodies recognize more epitopes on a mammalian protein than the corresponding rabbit doses, making it advantageous to use IgY in immunological assays of mammalian proteins. This is especially true the antigen is highly conserver protein, such as hormone (Gassmann et al., 1986).

Larson and Sjoquist (1988) reported that latex particles coated with chicken antibodies can detect $10^{-9}$ g of extracellular protein A per litre in the absence of serum and $10^{-7}$ g of protein A per litre in the absence of 10% normal human serum. The assay was performed within a minute.

Jensenics et al. (1981) suggested that chicken antibodies might also be of interest for developing other bacteriological assays when there is a risk of false positive results owing to reactions between Fc region of mammalian antibodies and staphylococcal protein A or streptococcal protein G.

Hatta et al. (1997) worked on Passive immunization against dental plaque formation in humans and effect of a mouth rinse containing egg yolk antibodies (IgY) specific to Streptococcus mutans and they reported the effectiveness of IgY with specificity to S. mutans grown in the presence of
sucrose as an efficient method to control the colonization of mutans streptococci in the oral cavity of humans.

Lee et al. (2000) reported the effects of hen egg yolk immunoglobulin in passive protection of rainbow trout against *Yersinia ruckeri*. They reported that feeding specific anti-serovar 1 *Yersinia ruckeri* IgY to fish either before or after immersion infection produced marginal reductions in mortalities and in intestine infection. The same IgY passively protected rainbow trout against infection when administered by intraperitoneal injection 4h before an immersion challenge.

Calzado et al. (2001) worked on Human haemoclassification by use of specific yolk antibodies obtained after immunization of chickens against human blood group antigens. They reported that the highest titres were observed four weeks after the first immunization, and these remained stable for up to seven weeks for the intravenous route. Positive reactivity against human erythrocyte antigens A, B and O was demonstrated in de-lipidated supernatants from the egg yolks of immunized hens. The strongest reaction was observed against blood group O Rh+ (O+).

Na Ri Shin et al. (2002) reported an effective method for the production of immunoglobulins (IgY) using immunogens of *Bordetella bronchiseptica*, *Pasteurella multoucla* and *Actinobacillus pleuropneumoniae*. The research suggests that new vaccines could be effective in the production of egg yolk antibodies against the causative agents of swine.

Peter Hodek et al. (2003) reported chicken antibodies as superior alternative for conventional immunoglobulins. Antibodies purified in large amount from egg yolks were found to be suitable for passive immunization against pathogenic microorganisms and toxins.

Gazim Bizanov et al. (2003) worked on production and purification of Igy from egg Yolk after immunization of hens with pig IgG and reported that the specific activity and IgY contents when purified by ammonium sulphate were 1.6-2.0 and 1.6-1.8-fold higher than those purified by the zinc sulphate or cadmium sulphate method.
Deog Yong Lee et al. (2004) developed a novel antigen capture-ELISA using IgY against porcine interleukin-6. The results suggested that the developed antigen capture-ELISA could be a good tool for the screening of microbial infections in pig farms.

Bizhanov et al. (2004) developed a novel method, based on lithium sulfate precipitation for purification of chicken egg yolk immunoglobulins (IgY), applied to immunospecific antibodies against Sendai virus. Here two IgY purification methods based on salt precipitation using lithium sulfate or sodium citrate were developed. These methods were compared with polyethylene glycol precipitation and chloroform extraction methods. The results indicate that the purification of IgY by lithium sulfate results in very pure IgY in high quantities.

Gholamreza Asadi Karam et al. (2005) extracted and purified antiproteinase 3 PR3 antibodies from egg yolk. He also reported that IgY binds neither to mammalian complement or Fc receptor nor does it interfere with Rheumatoid factors. Results also showed that the prepared IgY-anti-PR3 had good titer and specificity.

Shanmugasamy Malmarugan et al. (2005) reported an alternative source of antibody for diagnosis of infectious bursal diseases. The anti-infectious bursal disease-IgY thus harvested was found to possess immunodiagnostic potency as assessed by agar gel precipitation test and counter immunoelectrophoresis for replacing the use of conventional antibody.

Alexander et al. (2006) worked on preparation of anti-*Candida albicans* antibodies in an egg-laying hen and their protective efficacy in mice and reported that post-immunization IgY might be considered a prophylactic agent or possibly an adjunct to antifungal therapy. Pre-immunization IgY appeared to contain factors that prolonged survival, but did not prevent dissemination of the fungus.

Muhammad Wasif Malik et al. (2006) worked on passive immunization using purified IgYs against infectious bursal disease of chickens in Pakistan and reported that infectious bursal disease virus infected birds were injected with purified antibodies which induced 92% recovery as compared to control birds and also reported that the purified antibodies may be useful as a therapeutic agent to cure IBD infected birds.
Sa Van Nguyen \textit{et al.} (2006) reported passive protection of dogs against clinical disease due to Canine Parvovirus-2 by specific antibody from chicken egg yolk. The IgY treated groups had significantly greater weight gain and shorter duration of virus shedding that the control group. The results indicate that IgY is useful in protecting dogs from canine Parvovirus-2.

LI Xiao-liang \textit{et al.} (2006) reported the protection of \textit{Carassius auratus gibelio} against infection by \textit{Aeromonas hydrophila} using specific immunoglobulins from chicken egg yolk. The specific IgY inhibited the growth of \textit{A. hydrophila} at a concentration of 1.0 mg/ml during the 18 h incubation. The results showed that this alternative approach can control diseases in fishes caused by this organism.

Meenatchisundaram \textit{et al.} (2008a, b) reported that antivenom generated in chicken could be used for therapeutic purposes in case of snakebite envenomation.

Meenatchisundaram \textit{et al.} (2009) reported that the Freund’s adjuvant is the preferred adjuvant to generate chicken egg yolk antivenom antibodies to treat snake bite envenomations.

Shahbazi \textit{et al.} (2009) worked on specific egg yolk antibody against recombinant \textit{Cryptosporidium parvum} P23 protein and reported that since p23 is an immunodominant surface glycoprotein expressed in the early phase of infection, specific IgY against recombinant p23 could be recommended as a favourable candidate for passive immunization against \textit{C. parvum} infection in human and animals.

Lee \textit{et al.} (2009) worked on induction of passive immunity in broiler chickens against \textit{Eimeria acervulina} by hyperimmune egg yolk immunoglobulin Y and concluded that passive immunization of chickens with anti-coccidia IgY antibodies provide immunity against coccidiosis challenge infection.

Marco \textit{et al.} (2009) worked on growth inhibition of \textit{Staphylococcus aureus} by chicken egg yolk antibodies and the findings indicate anti-\textit{S. aureus} IgY obtained from hens immunized with \textit{S. aureus} ATCC 33593 may provide an interesting alternative to antibiotic use in the management of \textit{S. aureus} infectious in humans and animals.
Meenatchisundaram *et al.* (2010) reported that the purification of IgY by PEG and ammonium sulphate yielded very pure IgY at high quantities (93% ± 5% of total egg yolk protein), which was also capable of neutralizing toxic and lethal components of the *E. carinatus* venom.

Ma *et al.* (2010) worked on preparation of immunoglobulin Y (IgY) against lipopolysaccharide using gel chromatography from the yolks of egg laid by immunized hens and reported that this method provided an efficient way to produce high titer egg yolk antibodies, which could attenuate lethal effects of LPS, by immunizing hens. Furthermore, the LPS antibody was purified well using a water solution, salting out and gel chromatography.

Diraviyam *et al.* (2011a) worked on in vitro studies of chicken egg yolk antibodies generated against *Salmonella pullorum* and reported that the raised antibodies could be used for the passive immunotherapy to protect the young chicks from horizontal transmission of Pullorum disease by improving the immunological strength against infectious disease.

Diraviyam *et al.* (2011b) worked on preparation of chicken (IgY) antibodies consortium for the prevention of enteric infections in poultry and reported that the purified chicken antibodies can be used to prepare consortium for passive immunization to protect the young chicks from enteric infections.

Malekshahi *et al.* (2011) worked on treatment of *Helicobacter pylori* infection in mice with oral administration of egg yolk driven anti-UreC immunoglobulin and reported that UreC-induced IgY is specifically successful in inhibition of *Helicobacter pylori* infection and could be an alternative to antibiotic treatment.

Mulvey *et al.* (2011) worked on therapeutic potential of egg yolk antibodies for treating *Clostridium difficile* infection and reported that the egg yolk preparations obtained from chickens immunized with recombinant *Clostridium difficile* CFs may represent another safe and cost effective treatment option in humans suffering from acute or recurring CDI.

Neri *et al.* (2011) worked on specific egg yolk immunoglobulin as a new preventive approach for Shiga-toxin-mediated diseases and reported that anti-Stx IgY antibodies may be considered as preventive agents for Stx-mediated diseases in EHEC infection.
Parma et al. (2011) worked on antibodies anti-Shiga toxin 2 B subunit from chicken egg yolk: isolation, purification and neutralization efficacy and reported that immunization of hens with Stx2B could be a strategy to obtain at low cost a relatively high concentration of anti-Stx2 egg yolk IgY, able to neutralize Stx2 lethal activity. IgY technology could be an useful tool for research, diagnosis and therapy of EHEC infection.

Pauly et al. (2011) worked on IgY technology: extraction of chicken antibodies from egg yolk by polyethylene glycol (PEG) precipitation and reported that the laying capacity of a hen per year is around 325 eggs. That means a total potential harvest of 20 g total IgY/year based on a mean IgY content of 60 mg total IgY/egg.

Sui et al. (2011) worked on antibacterial activity of egg yolk antibody (IgY) against Listeria monocytogenes and preliminary evaluation of its potential for food preservation and reported that he results suggest the potential application of specific IgY as a natural antimicrobial agent for food preservation.

Ali (2011) worked on effect of immunoglobulin Y purified from immunized hen eggs on the growth of Staphylococcus aureus and reported that the IgY purified by agar- PEG method, obtained from hens immunized by formalin treated S.aureus, showed a significant reduction in bacterial growth and the growth inhibition was depended on specific IgY concentration and may provide a novel approach to the management of S. aureus infections.

Vega et al. (2011) worked on egg yolk IgY: protection against rota virus induced diarrhoea and modulatory effect on the systemic and mucosal antibody responses in new born calves underreported that strong active ASC immune response is induced in the intestinal mucosa following BRV infection after the administration of egg yolk, regardless the specificity of the treatment.

Zhen et al. (2011) worked on efficacy of specific IgY for treatment of lipopolysaccharide-induced endotoxemia using a mouse model and reported that the specific IgY increased the survival rate of mice with endotoxemia induced by LPS, down-regulated TNF-α and up-regulated IL-10 in serum and attenuated the extent of damage to the lung and liver.
Meenatchisundaram *et al.* (2011) worked on development of chicken egg yolk antibodies against *Streptococcus mitis* – purification and neutralizing efficacy and reported that the antibodies generated in chicken could be used for diagnosis and therapeutic purposes in case of *Streptococcus mitis*.

Ya Fu Xu *et al.* (2011) worked on production and characterization of egg yolk antibodies (IgY) against two specific spoilage organisms (SSO) in aquatic products and results indicated a great potential of specific IgY as a safe and natural antimicrobial agent for aquatic food preservations.

Cai *et al.* (2012) worked on chicken egg yolk antibodies (IgY) for detecting circulating antigens of *Schistosoma japonicum* and reported that the developed immunoassay is reasonably sensitive and specific. It could be used for field research and treatment efficacy assessments.

Gholamreza Nikbakht *et al.* (2012) worked on the generation of egg yolk antibodies in chicken (IgY) against Influenza M2 (M2e) protein and the result shows that the anti-M2e polyclonal, monospecific IgY antibodies could be used for different areas of research, diagnostics, medical application and biotechnology.

Gujral *et al.* (2012) worked on in-vitro and in-vivo binding activity of chicken egg yolk immunoglobulin Y (IgY) against gliadin in food matrix and reported that EYP-M containing IgY antibody may be used in CD patients to eliminate the effects of ingested toxic gliadin.

Kovacs-Nolan *et al.* (2012) worked on egg yolk antibodies for passive immunity and reported that the IgY is used for passive immunization to treat and prevent human and animal diseases.

Michael *et al.* (2012) worked on the generation and characterization of chicken egg yolk antibodies against *P. acnes* for the prevention of acne vulgaris and the findings indicate that anti-acne IgY is worth utilizing as a preventive agent for acne vulgaris.

Nilsson *et al.* (2012) worked on IgY stability in eggs stored at room temperature or at +4°C and reported that the eggs from individual hens were randomised and stored for up to one month at room temperature, or for up to 6 months at +4°C. IgY was extracted from the egg yolks and the antibody activities were tested by ELISA and there was no significant reduction in antibody titres with egg storage under these conditions.
Vaillant et al. (2012) worked on the chicken and egg system for the development of anti-idiotypic vaccines and the results of this study suggest that eggs from immunized hens could be considered in the management of HIV infections.

Wen et al. (2012) worked on preparation and characterization of egg yolk immunoglobulin Y specific to influenza B virus and reported that the that IgY is an easily prepared and rich source of antibodies that offers a potential alternative strategy for preventing and treating influenza B infections.

Xiaoyu Li et al. (2012) worked on chicken egg yolk antibody (IgY) controls Solobacterium moorei under in vitro and in vivo conditions and this study demonstrates that the growth and biofilm formation of S. moorei can be effectively inhibited by specific IgY. As a result, IgY technology may have application in the control of diseases caused by S.moirei.

Bo-Mi Kim et al. (2012) worked on efficacy of specific immunoglobulin egg yolk (IgY) against enterotoxigenic Escherichia coli K99, Salmonella typhimurium and Salmonella choleraesuis and the results indicate the potential of specific IgY for the treatment of porcine bacterial disease caused by E.coli K99, S.typhimurium and S.choleraesuis.

Ferella et al. (2012) worked on chicken egg yolk antibodies against bovine respiratory syncytial virus and reported that the purified IgY against BRSV was able to neutralize the virus in a virus neutralization assay and the results suggests the potential use of IgY as a prophylactic treatment against RSV infection.

Xu et al. (2012) evaluated the effectiveness of egg yolk immunoglobulin (IgY) against periodontal disease-causing Fusobacterium nucleatum and reported that the IgY effectively inhibited growth and biofilm formation by F. nucleatum and prevented the progression of periodontal disease by decreasing alveolar bone loss. Thus specific IgY may have potential for the treatment of periodontal disease.
Kim et al. (2013) worked on the potential to reduce poultry nitrogen emissions with specific uricase egg yolk feed grade antibodies and reported that egg yolk antibodies are economic alternatives for administering in feed to poultry. Supplementation of feed grade antibodies into poultry diets can be a potential approach to minimise bacterial uricase activity and reduce ammonia emissions from poultry manure.

Fábio Goulart de Andrade et al. (2013) worked on the production and characterization of anti-bothropic and anti-crotalic IgY antibodies in laying hens and showed that the administration of successive doses of the venoms for more than 6 months results in an antivenin with higher avidity that is able to recognize a greater number of antigens present in the venoms. These characteristics indicate a more efficient and potent antivenin than what has been described in other studies.

Aurora Alvarez et al. (2013) worked on IgY antibodies anti-*Tityus carpitensis* venom: Purification and neutralization efficacy and reported that the anti- venom was effective in neutralizing 2LD$_{50}$ doses of *T. carpitensis* venom (97.8 mg of IgY neutralized 1 mg of *T. carpitensis* venom). These results support the future use of avian anti-scorpion venom as an alternative to conventional equine anti- venom therapy in worldwide.

Oloyede and Faparusi (2013) worked on characterisation of antibodies from egg yolk of some birds and reported that egg yolks of immunized bird and local birds are good sources of immunological active IgG antibody.

Aaydh et al. (2013) worked on avian antibodies for staphylococcal enterotoxin B as an efficient tool for FRET-Based Fluoroimmunosensor reported that bioconjugation of nanoparticles demonstrated their efficiency in sensitive monitoring of staphylococcal enterotoxin B (SEB) through immuno-affinity reactions to address the potential health risk and economic impacts of staphylococcal food poisoning.

Salma et al. (2013) worked on production and evaluation of chicken egg-yolk-derived antibodies against *Campylobacter jejuni* colonization-associated proteins and reported that these α-*C. jejuni* colonization-associated proteins (CAP)-specific IgY may be useful as a passive immunotherapeutic to reduce *C. jejuni* colonization in chickens.
Dinesh et al. (2013) worked on characterization and in vitro neutralization of *Streptococcus mutans* egg yolk antibodies (IgY) and the results indicated that chicken IgY could be used for diagnosing dental caries caused by *Streptococcus mutans* and as a therapeutic agent.

Karthika et al. (2013) worked on Immunocosmeceuticals: An emerging trend in repairing human hair damage and they introduced with an active ingredient comprising a yolk derived anti-hair antibody immunoglobin obtained from egg of chickens immunized with damaged hair as antigen. This immunocosmeceuticals can repair the hair damage and imparts flexibility and smoothness to the hair. These effects are not lost by the ordinary shampooing.

Sitnik et al. (2013) worked on production and characterization of egg yolk antibodies against bovine alimentary tract pathogens and reported that all used vaccines induced the rise of IgY antibody in egg yolks. Based on the duration and the highest level of IgY antibody against bovine alimentary tract pathogens C vaccine was further used in next two trials for vaccination of 1000 hens each time. Double immunization seems to be enough in mounting response against examined pathogens for several weeks. Immunization with C vaccine allowed harvesting eggs with satisfactory levels of E.coli, rotavirus and coronavirus IgY antibodies which may be used to evaluate their protective effect by oral administration in calves.

Liji Jin et al. (2013) worked on protection of crucian carp (*Carassius auratus Gibelio*) against septicaemia caused by *Aeromonas hydrophila* using specific egg yolk immunoglobulins and the results suggests that passive immunization by immersion with pathogen-specific IgY may provide a valuable treatment for *A. hydrophila* infection in carp.

Kuncorojakti and Suwanno (2013) worked on production and characterization of egg yolk derived anti-hemaglutinin antibody (IgY) as immunotherapy agent on the chicken infected by avian influenza A/H5N1 virus and concluded that the anti-HA antibody both derived from chicken egg yolk and blood sera can bind to the antigen of Avian influenza A/H5N1 virus.
Megha et al. (2014) worked on generation and characterization of specific chicken egg yolk antibodies (IgY) against microbial bio-terroristic agent (*Vibrio cholera*) and reported that the specific IgY antibodies generated against *V. cholerae* were known to have convincing reactivity and specificity against the antigen. This chicken egg yolk IgY produced can be applied for passive immunotherapy, diagnosis and in detection kits during a microbial bioterroristic attack.

Luzia et al. (2014) worked on in vitro cytotoxicity and genotoxicity of chicken egg yolk antibodies (IgY) against *Trypanosoma evansi* in human lymphocytes and reported that IgY antibodies anti-*T. evansi*, at concentrations of 1, 2.5, 5 and 10 mg/mL did not cause damage to the cell membrane of human lymphocytes, did not affect the cell viability, as well as did not produce DNA damage at the chromosomal level; thus, not presenting cytotoxicity and genotoxicity. These findings demonstrate the safety of these antibodies to mammalian cells.

Revathy et al. (2014) worked on in vitro evaluation of the efficacy of chicken egg yolk antibodies (IgY) generated against *Propionibacterium acnes* and concluded that the specific antibodies developed against *P. acnes* were found to be effective and they can be used in the acne therapy in the form of topical creams with further research work to prove their efficacy in vivo. Being cost efficient, they appear to be a promising alternative to the current antimicrobial therapy.

Hou et al. (2014) worked on the Protective effect of an egg yolk-derived immunoglobulin (IgY) against *Prevotella intermedia*-mediated gingivitis and developed a new immunoglobulin specific to *P. intermedia* from egg yolk. This specific IgY can dose-dependently inhibit the growth of *P. intermedia* and protect rats from gingivitis induced by *P. intermedia*. The new IgY has potential for the treatment of *P. intermedia*-mediated gingivitis.
Chang Hong Li et al. (2014) studied passive protective effect of chicken egg yolk immunoglobulins against experimental *Vibrio anguillarum* infection in ayu (*Plecoglossus altivelis*) and reported that the phagocytic activity of macrophages for *V. anguillarum* in the presence of specific IgY was significantly higher than that seen for nonspecific IgY. These results suggest that passive immunization by oral intubation with pathogen-specific IgY may provide a valuable treatment for *V. anguillarum* infection in ayu.

Cecilia et al. (2014) worked on egg yolk antibodies (IgY) against bovine leukemia virus and the results suggests that chicken IgY may be a suitable platform to produce large amounts of anti-BLV antibodies for diagnostic systems. Furthermore, the use of IgY for passive immunization against BLV infection should also be explored in order to develop new strategies to control the disease in cattle.

Ya-Jie Sheng et al. (2014) worked on production of chicken yolk IgY to sulfamethazine: comparison with rabbit antiserum IgG and the results indicated that the IgY potentially provides a practical and ethical alternative to IgG in veterinary drug residue immunoanalysis.

Baloch et al. (2014) reviewed IgY technology in aquaculture and concluded that IgY antibodies have been developed for the treatment and prevention of certain fish diseases such as white spot syndrome in shrimp, vibriosis, enteric redmouth disease and edwardsiellosis. This review presents an analysis of the characteristics, extraction methods, therapeutic, detection, and seafood-persevering applications of IgY antibodies in aquaculture.

You et al. (2014) worked on chicken egg yolk immunoglobulin (IgY) developed against fusion protein LTB–STa–STb neutralizes the toxicity of *Escherichia coli* heat-stable enterotoxins and concluded that the genetically constructed Bab induced significant antibody responses against STa and STb in chickens, and the resulting IgY had the capacity to neutralize the toxicity of ST. The recombinant Bab protein containing three important ETEC enterotoxins may serve as an effective and convenient polyvalent toxoid that can be used to produce multiple antitoxin IgYs to prevent colibacillosis caused by ETEC with various fimbriae in young animals.
2.17. Chicken Antibodies against Mastitis Pathogens

Zhen et al. (2008a) reported that the specific IgY against mastitis-causing *Staphylococcus aureus* inhibited the growth of *S. aureus* and enhanced the phagocytosis of *S. aureus* by milk macrophages. He also reported that the specific IgY against mastitis-causing *Staphylococcus aureus* inhibited the growth of *S. aureus* and enhanced the phagocytosis of *S. aureus* by milk macrophages.

Zhen et al. (2008b) worked on characterization of specific egg yolk immunoglobulin (IgY) against mastitis causing *Escherichia coli* and they concluded that the growth inhibition activity of specific IgY to bacteria was dose-dependent with an effective concentration of 20mg purified IgY per millilitre. The phagocytic activity of *E.coli* either by milk macrophages or by polymorphonuclear neutrophil leucocytes in the presence of specific IgY was significantly higher than that with non specific IgY or without IgY. These results suggest that this specific IgY has potential as a therapeutic treatment for mastitis in dairy cows.

Wang et al. (2011) studied characterization of chicken egg yolk immunoglobulins (IgYs) specific for the most prevalent capsular serotypes of mastitis-causing *Staphylococcus aureus* and reported that all of the specific IgY significantly blocked the internalization of their homologous strains by bovine mammary epithelial cells.

Meenatchisundaram et al. (2011) worked on Isolation, Purification and Neutralizing potential of chicken egg yolk immunoglobulin (IgY) against mastitis causing *Escherichia coli* in dairy cows in Coimbatore District and the results indicated that the antibodies generated in chicken could be used for diagnostic and therapeutic purposes in case of bovine mastitis.

Uma et al. (2012) worked on purification and characterization of chicken egg yolk antibodies (IgY) against mastitis causing *Klebsiella pneumoniae* and the results indicated that antibodies generated in chicken effectively neutralized mastitis causing *K.pneumoniae* and has a potential application in diagnosis and treatment of mastitis causing *K.pneumoniae*. 
Gabriel Leitner et al. (2013) studied immunotherapy of mastitis in which he evaluated the efficacy of a microbead carrying specific anti-mastitis bacteria antibodies and an enhancer of phagocytosis, termed Y-complex, in treating cows infected by mastitis bacteria and reported that the efficacy of Y-complex in treating major mastitic pathogens like *E.coli* and *S.dysgalactiae* was similar to that of antibiotics. Thus the Y-complex was proven to be efficient and may serve as a new approach for the treatment of mastitis.

Mahenthiran et al. (2013) worked on generation, purification and neutralization potential of chicken egg yolk antibodies (IgY) against mastitis causing *E.coli* and *Staphylococcus aureus* and reported that the antibodies generated were potent enough to inhibit the growth of *E.coli* and *S.aureus*. These highly purified chicken egg yolk antibodies could be used to treat bovine mastitis, economically important disease hampering desired progress in the dairy industry and play an increasing role in research, diagnostics and immunotherapy in future.

Iqbal et al. (2013) studied *In vivo* comparison of specific activity of egg yolk immunoglobulins (IgY) and antibiotics against *Staphylococcus aureus* causing mastitis in buffaloes (*Bubalus bubalis*) and found that the milk yield of 90% and 40% buffaloes was found increased in the groups that received egg yolk antibodies and antibiotic, respectively. Similarly, clinical and microbiological cures rates were 50% better in the egg yolk treated buffaloes than antibiotic treated buffalo groups.