2. Prevalence of *Salmonella* in Eggs of Poultry, Duck and Japanese quail

2.1. Introduction

Salmonellosis is one of the most frequently occurring food borne illness resulting from the ingestion of viable cells of a member of the genus *Salmonella*. Eggs, poultry, meat and meat products are reported to be the most common food vehicles of salmonellosis to humans (Oosterom, 1991; Jay, 1992; Davies and Wray, 1996). Human illness caused by infection with *Salmonella* spp. increased worldwide beginning as early as the mid 1970’s. As per the statistics available at the end of 20th century it has been reported that Germany, Austria and Poland experienced 100-200 cases of *S.* Enteritidis salmonellosis per 100000 people, whereas England peaked at 40 cases and France at 10 cases per 100000 people (Gomez, 1997). Owing to the wide spread occurrence of *Salmonella* food poisoning, considerable attention has been given in recent years to destruction or control of the responsible microorganism.

2.2. Review of Literature

2.2.1. Poultry and *Salmonella*

In humans, as in animals, salmonellosis is primarily a disease confined to the gastrointestinal tract, but may cause serious extra-intestinal tract disease (Wilkins and Roberts, 1988; Sechter *et al.*, 1991). There is a link between salmonellosis in poultry and people. Poultry and poultry products contaminated with *Salmonella* frequently leads to salmonellosis in humans (Lester *et al.*, 1990; Olsen *et al.*, 1992; Morse *et al.*, 1994). The enormous production of broilers is reflected in the nation’s consumption of meat which now comprises more poultry and less beef and mutton than previously. Contamination of broiler carcasses with human enteropathogens remains a problem of the broiler industry, food safety regulation agencies, and concerned consumers (Corrier *et al.*, 1999; Wedderkopp *et al.*, 2001; Vandeplas *et al.*, 2008). Those
serovars and phage types that are most prevalent in poultry are also commonly isolated from people (Rowe and Lior, 1980; Rampling et al., 1989; Telzak et al., 1990; Altekruse et al., 1993; Poppe, 1994).

The latest data on poultry contamination compiled by the USDA's Food Safety and Inspection Service shows about 16 percent of poultry tested positive for Salmonella. The highest rates of contamination were reported in ground turkey and broiler chickens (Howard News Service, 2006). A study conducted by Sean et al. (2006) in United States found that there is significant increase in the number of broiler chicken slaughter establishments with S. Enteritidis. The Centers for Disease Control estimates that 1.4 million people get sick from Salmonella in the United States each year, with about 400 deaths (Howard News Service, 2006). However, thorough cooking kills the bacteria and makes the food harmless.

It has been well documented that poultry constitutes the largest single reservoir of salmonellae and that the non-host adapted serovars pathogenic to poultry are also potential pathogens of man and other animals (Verma and Gupta 1997; Rabsch et al., 2002). Isolation of salmonellae from poultry has been regularly reported in India (Gupta and Verma, 1989). Gupta and Verma (1990) isolated 528 Salmonella isolates from the farm animals which were typed at National Salmonella Centre (Veterinary) (NSCV) during 1976-89. Their findings revealed that poultry constituted the major animal source of salmonellae followed by sheep/goat, pig, equine and cattle/buffalo.

It has been reported that Salmonella infections in humans have increased worldwide and continue to be a major public health concern (Rodrigue et al., 1990). In the United States and in the United Kingdom this increase has been dramatic. In United States 51% of human Salmonella isolates were S. Enteritidis. (Madden 1989). S. Enteritidis and its association with egg and egg products have had the most
significant impact on the incidence of human salmonellosis (Cowden et al., 1989; Stevens et al., 1989; Luby and Jones, 1993). During the last 5-10 years in European countries and the United States, human S. Enteritidis infections have increased to such an extent that S. Enteritidis has become the most commonly isolated Salmonella serovar. Many other countries have also experienced outbreaks of human salmonellosis caused by S. Enteritidis, identifying the consumption of eggs or foods containing eggs as the major vehicle of transmission (Perales and Audicana, 1989; Rodrigue et al., 1990; van der Giessen et al., 1992; Wong et al., 1994; Cox 1995; Julian et al., 2002).

A study on the prevalence of S. Enteritidis and other Salmonella species in Canadian registered commercial chicken broiler flocks by Poppe et al., 1991 reported that 76.9% of randomly selected flocks were contaminated with salmonellas. The most prevalent serovars were S. Hadar and S. Schwarzengrund. Another study by Khakhria (1991) in Canada observed that S. Enteritidis isolation from humans rose from 8.3% in 1987 to 9.2% in 1988.

In Europe, most human S. Enteritidis isolates belonged to phage type (PT) 4, whereas in Canada and the United States they were mostly PT8 (Poppe et al., 1991). In 2001 Denmark witnessed a major reduction in the incidence of food borne human salmonellosis that has been achieved by ‘Salmonella control programme’. In the same year, the Danish Society saved U.S$25.5 million by controlling Salmonella. Hansen (1970) reported that in Denmark S. Typhimurium and ‘O’ form are responsible for most outbreak of salmonellosis among hens, ducks and gheese. Rosa et al. (2003) conducted a study to examine the incidence of Salmonella in Spanish poultry products. S. Enteritidis, S. Poona, S. Paratyphi B and S. Worthigton were isolated in 34.3%, 11.4%, 2.8% and 1.4% of the samples respectively. Berghold et al. (2004) reported that in 2002, S. Enteritidis phage type 29 (PT29) was first isolated from non-human samples in Austria. In England and Wales, isolation of S.
Enteritidis from humans increased from 1087 in 1981 to 1547 in 1989, whereas infections due to all other serovars combined increased by about half (Cowden et al., 1989; Frost et al., 1989). Similarly, in Scotland, S. Enteritidis infections in humans increased from 11% of all isolates of Salmonella in 1982 to 52% in 1988 (Sharp, 1989). In Spain, outbreaks of food-borne disease in humans due to S. Enteritidis increased from 8% in 1977 to 40% in 1984 (Peraltes et al., 1988).

Elvira et al. (2001) isolated 11 Salmonella strains from turkey meat samples in Albania during 1996-1998. The most frequently encountered serotypes in this study were S. Enteritidis and S. Agona. An important finding of this investigation was the isolation of S. Blockley, one of the most common isolated serotype from both human and avian sources in the Far East countries.

Mieczy Radkowski (2001) conducted a study on the occurrence of Salmonella spp. in consumption egg in Poland. Salmonella was not found on the shell or inside the egg. The findings give the impression that eggs are relatively rarely contaminated by Salmonella species on the surface. However, in this study only a small number of eggs are examined in proportion to the number of eggs laid.

Shell eggs (Humphrey, 1990) and poultry meat products (Poppe et al., 1991) were regarded as important sources of human salmonellosis and it is essential to reduce the level of contaminated material entering the human food chain to avoid food borne infections and consequent economic losses (Roberts, 1989; Todd, 1989; Bender and Ebel, 1992; Bailey, 1993). As the consumption of chicken is steadily increasing in India, it will be worthwhile to assess the level of Salmonella contamination in chicken, with special reference to S. Enteritidis.

In most of the countries, infections of humans with S. Enteritidis has often been associated with the consumption of eggs, foods that contain eggs, poultry meats
and other poultry products contaminated with S. Enteritidis (Rampling et al., 1989; Telzak et al., 1990; Altekruse et al., 1993). From 1998 through 2006, four outbreaks of salmonellosis associated with raw, frozen, microwaveable, breaded, prebrowned, stuffed chicken products were identified in Minnesota (Smith et al., 2008).

2.2.2. Penetration of *Salmonella* through egg shell

The estimation and identification of microorganisms on the eggshell and its contents are of great importance to the poultry industry, since the contamination and subsequent penetration of the egg shell by bacteria has public health significance (Arhienbuwa et al., 1980). Methods for studying microbial penetration through the outer structures of the avian egg have been established since the late 1960's (Williams and Whittemore, 1967). Although, the possible chance of congenital infection of eggs cannot be ruled out, major contamination of eggshell occurs after the eggs are laid and eventually the bacteria penetrate the shells (Lorenz et al., 1952; Harry, 1963). The extent to which such contaminants may bring about undesirable changes in egg contents depends primarily upon the ability of these bacteria to overcome the antibacterial properties of the albumin (Lutsky and Bell, 1953) and their ability to utilize protein complexes of egg indicating a fundamental relationship between egg spoilage and the composition of bacterial flora of the egg shell (Harry, 1963). Messens et al. (2005) reviewed the penetration of *Salmonella* through the shell of the egg and indicated that there is a need to investigate both the overall incidence of *Salmonella* in layer flocks, and to determine the significance of prevalent serovars, especially those that have been associated previously with poultry and, as a consequence, human disease.

2.2.3. The predominance of various serotypes of *Salmonella* in poultry

The predominance of various serotypes in different periods was reported. A particular serotype, which was considered as an important contaminating agent
during a period, was replaced by some other serotype in another period. Poppe et al. (1991) reported that contamination of broiler chickens with various serotypes of *Salmonella*. Contamination in chicken meat with various serotypes of *Salmonella* has been also reported by Rose et al. (1990) and Gorman et al. (2002). The prevalence of *S*. Typhimurium has decreased steadily and *S*. Enteritidis has become the most prevalent serovar in poultry and in people (Rodrique et al., 1990; Qin et al., 1995). In an outbreak of salmonellosis recorded by Rahiman et al. (1997), two *Salmonella* serovars namely *S*. Gallinarum and *S*. Indiana were isolated. *S*. Typhimurium associated outbreak in layers, was also recorded by Rehman et al., 2002.


A number of studies have found *S*. Infantis to be a prevalent serovar within the poultry industry worldwide. A large *S*. Infantis epidemic in broiler chickens associated with high incidence of human salmonellosis in Finland during 1975 implicated contaminated feed as a possible source of the bird infection (Raevuori et al., 1978). *S*. Infantis was also reported as the second most common serovar isolated from layer flocks in Canada in 1991 (Poppe et al., 1991). In Germany, *S*. Infantis was shown to be a prevalent serovar in layer flocks, second only to *S*. Enteritidis (Hinz et al., 1996).
2.2.4. Cross contamination of *Salmonella* in processing environment

Salmonellae are widely distributed in nature and cause a spectrum of diseases in man and animals. So far, as many as 2435 serovars of *Salmonella* have been reported (Popoff and Le Minor, 1997). Of these, 209 serovars of *Salmonella* have been reported from India and *S*. Typhimurium was found to be one of the most common serovars prevalent both in man and animals. The transmission of *S*. Enteritidis infections to humans by contaminated eggs has been a prominent food safety issue throughout much of the world for more than a decade (Angulo and Swerdlow, 1999; Centers for Disease Control, 2000). Contamination can occur at multiple steps along the food chain including production, processing, distribution and retail marketing (Shaohua et al., 2003). Stephens et al. (2008) reported the outbreaks of *S*. Typhimurium in Tasmania are due to the inadequate food safety practices linked with common poultry farms.

Numerous studies have focused on the reduction of *Salmonella* colonization in poultry and the prevention of carcass contamination during processing (Izat et al., 1990; James et al., 1992; Waldroup et al., 1992; Hargis et al., 1995). Contaminated chicken meat and eggs have been identified as the most frequently implicated vehicles to *S*. Enteritidis (Schmidt, 1995). Minimization of bacterial contamination is of major interest among poultry processors and food safety researchers, because of the frequently incriminated poultry products with *Salmonella* such as poultry flocks (Kardel and Miller, 1991; Baggesen et al., 1992), shell eggs (Humphrey, 1990) and poultry meat products (Poppe et al., 1991). More over, most available evidence suggests that egg contamination by *S*. Enteritidis typically involves relatively small initial numbers of bacterial cells (Humphrey et al., 1991; Gast and Holt, 2000). Simmons et al. (2003) reported *Salmonella* positive carcasses samples from processing plants. It is clearly demonstrated that the reduction of microbial contamination of poultry meat products...
required the identification of both pre-harvest and post-harvest critical control points where contamination may occur, and the implementation of integrated control programs (Rigby and Pettit, 1980; Goren et al., 1988; Jones et al., 1991; Blankenship et al., 1993; Strauric and D’Aoust, 1993).

It was reported that S. Enteritidis PT4 was able to cross contaminate beef through contaminated egg droplets present on the processing surface (Bradford et al., 1997). In another investigation on Salmonella, it was found that under cooking and cross contamination of cooked foods from raw chicken are the major causes of infections related to chicken products (Boer and Hahne, 1990). Reduction in the total numbers of Salmonella cells colonizing chicken as well as reduction in the number of infected chicken entering the processing plant would likely result in decreased carcass cross-contamination during processing (Hume et al., 1995). Cross-contamination during processing frequently results from the rupture of digestive organs during evisceration and subsequent immersion of carcasses in common chill bath (James et al., 1992). Prolonged survival of Salmonella outside living host was reported (Davis and Wray, 1995). Salmonella is less sensitive to drying and could be isolated from artificially contaminated surfaces up to 6 hours after the surface had dried (Dewit et al., 1979). It has been reported that raw meat and particularly poultry meat were important in the dissemination of potential human pathogens including Salmonella in the kitchen utensils, work surfaces and hands (Humphrey et al., 1994). Little et al. (2006) conducted a survey in Non-UK, and found out that in most of the contaminated egg samples, Salmonella was found to be on, rather than in, the egg; out of the 157 Salmonella positive shell samples, 10 of them were found to have salmonellae in the contents. There is an obvious cross-contamination risk from this external contamination, in that the organism can be transferred to other surfaces (e.g. hands) or to the egg contents during breaking of the shells. The presence of Salmonella on the surface of the shells of eggs therefore represents a potential risk to public health, just as well as
contamination of the contents of the egg (Little et al., 2006). Surface contamination may be the result of either infection of the lower reproductive tract of hens or faecal contamination (Humphrey, 1994; de Buck et al., 2004).

2.2.5. Cross contamination from cutting board

_Salmonella_ contamination on cutting board surfaces were reported (Ak et al., 1994). Survival of _Salmonella_ was reported on metal surface (Halke and Wong, 1994) and on plastic surfaces (Ak et al., 1994). It was reported that the concern with cutting boards at least in home kitchens is that bacteria of animal origin may cause cross-contamination (Ak et al., 1994). Fluid ("juice") from raw meat or poultry remaining on the work surface might transfer disease agents to other foods that would not be cooked further before being eaten. They also reported that bacteria of greatest concern as cross-contaminants on kitchen cutting boards were principally of animal origin but were significant causes of human infectious disease transmitted via foods and able to multiply at room temperature or below.

The transmission and survival of enteric pathogens in the food processing and preparation environment through human and raw food sources is reviewed by Todd, et al. (2009) with the main objective of providing information critical to the reduction of illness due to foodborne outbreaks. Pathogens in the food preparation area can originate from infected food workers, raw foods, or other environmental sources. These pathogens can then spread within food preparation or processing facilities through sometimes complex pathways and may infect one or more workers or the consumer of foods processed or prepared by these infected workers. The most frequent means of worker contamination is the fecal-oral route, and study results have indicated that toilet paper may not stop transmission of pathogens to hands. However, contact with raw foods of animal origin, worker aerosols (from sneezes), vomitus, and exposed hand lesions also have been associated with outbreaks.
Transfer of pathogens has been documented through contaminated fabrics and carpets, rings, currency, skin surfaces, dust, and aerosols and though person-to-person transmission. Results of experiments on pathogen survival have indicated that transmission depends on the species, the inoculum delivery route, the contact surface type, the duration and temperature of exposure, and the relative humidity. Generally, viruses and encysted parasites are more resistant than enteric bacteria to adverse environmental conditions, but all pathogens can survive long enough for transfer from a contaminated worker to food, food contact surfaces, or fellow workers.

2.2.6. Cross Contamination through litter feed and water

Litter samples have been reported to be best sampling sites and contamination in them was regarded as indication of Salmonella incidence in flocks (Kingston, 1981). Poppe et al. (1991) found contaminated feed and water in poultry rearing environments. Muhlenberg (1992) investigated that samples of chicken feed and litter are the primary source of Salmonella infection. S. Enteritidis and S. Heidelberg were identified from litter samples, serum samples and ovaries in the national poultry health programme of Alberta Agriculture, Food and Rural Development (2005). Ha et al. (2000) found out that poultry feed is a potential route for introduction of salmonellae into poultry production. Salmonella serotypes were isolated from feed samples (Goren et al., 1988) and insect samples (Jones, 1990). Corrier et al. (1999) suggested the presence of Salmonella in the crop and ceca of broiler chicken before and after preslaughter feed withdrawal. It was reported that the isolation of Salmonella from the crops of broilers at a commercial rearing house was greater after 8 hours feed withdrawal than before feed withdrawal (Ramírez et al., 1997).

2.2.7. Transmission of Salmonella through reproductive tract and feed

Egg contamination by S. Enteritidis is to occur before deposition of the shell (Humphrey et al., 1994; Methner et al., 1995; Gast and Holt, 2000; Richard and Jean
2004; Maddadi et al., 2010) by internal transmission to the contents of the egg via reproductive tract. An investigation on the prevalence of *Salmonella* in eggs observed that *S. Enteritidis* isolated from the yolk and albumen of eggs lay by naturally infected hens (Humphrey et al., 1989). Recovery of *S. Enteritidis* from inoculated pools of egg contents were reported (Gast, 1993). The reports showed that the infected hens can deposit *S. Enteritidis* in the yolk or albumen of developing eggs (Humphrey et al., 1989, 1991; Gast and Beard, 1990; Bichler et al., 1996; Gast and Holt, 2000). It has been reported that the effects of laying season and egg cracks on the ability of egg albumen to support the growth of *S. Enteritidis* in eggs (Yukiko et al., 2001). They reported that the growth of *S. Enteritidis* preferably in albumen of cracked eggs than intact eggs. Poppe et al. (1991) found out that *S. Enteritidis* may infect eggs by transovarian transmission.

### 2.2.8. *Salmonella* contamination in quail eggs

Due to increasing interest in Quail (*Coturnix coturnix japonica*) farming, scientists have been paying a lot of attention towards the prevention of different diseases of quails. Japanese quail has been recognized as an important laboratory animal because of its small body size, early maturity and the ability to produce several generations in a year (Anthony et al., 1996). The use of quail meat is also becoming increasingly important in many countries like France, Italy, Spain and Greece.

The report on *Salmonella* infection in quails seems to be very scanty. An outbreak due to *S. Typhimurium* (Sah et al., 1987), *S. Bareilly* (Kapoor et al., 1990), *S. Gallinarum* (Sarma et al., 1988; Mathew and Sulochana, 1990) *S. Enteritidis* (Kahramanmara, 2002) in liquid whole egg, are few reports of *Salmonella* contamination in Quails in India. Alexakis (1970), who isolated *S. Halle* from intestinal tract of apparently healthy quails, reported that Quail may also serve as a

Higher prevalence of *S.* Gallinarum has been reported in chicken (Gupta and Verma, 1989) and quails (Sarma *et al*., 1988; Mathew and Sulochana, 1990) causing heavy mortality both in young and adult resulting into heavy economic loss. The presence of *S.* Enteritidis in liquid whole quail eggs was investigated by Kahramanmara (2002).

### 2. 2. 9. Prevalence of *Salmonella* in India

Prevalence of various *Salmonella* serotypes has been periodically reported from man, animals, foods of animal origin and also other sources (Ganguli 1958; Hatha *et al*., 1997; Sharma *et al*., 1995). A study conducted by Saikia *et al.* (1986) from Assam reports the isolation of *S.* Chester and its monophasive variants for the first time in Assam. Verma *et al.* (2000) serologically identified 193 *Salmonella* cultures belonging to 29 serovars during the period 1994-96 from various sources at different places in India. These were isolated from various sources and different places of the country. All the strains belonged to sub species enterica. The first report of outbreak of salmonellosis associated with *S.* Enteritidis in ducklings in North East India reported by Rahman *et al.* (1999).

Anand *et al.* (1993) from Central Avian Research Institute, Izatnager, conducted a study on ‘Spoilage and pathogenic microflora of fresh chicken eggs’ in which samples were microbiologically examined for total aerobic counts along with the presence of some food borne pathogens such as *Salmonella* spp., coliforms, *Staphylococcus aureus*, *Bacillus cereus* and yeast and moulds. While none of the samples were found to be positive for *Salmonella* species, the other organisms were invariably encountered.
Review of literature clearly highlights egg as an important vehicle of food borne salmonellosis. Considering the fact that egg is highly nutritious and relatively inexpensive food item, the popularity of this commodity is increasing among the Indian population. Apart from that NECC is taking concerted efforts to promote egg as a complete food, which has also resulted in increased consumption of eggs. In this background it is important to assess the risk associated with this popular food item, especially in terms of presence of pathogen such as Salmonella. Hence the present study has been taken up with following objectives.

2.3 Objectives

1. To determine the prevalence of Salmonella in the contents of different types of eggs such as that of commercial layer hen, non commercial layer hens, duck and Japanese quail.
2. To determine the prevalence of Salmonella on the egg shell of different types of eggs such as that of commercial layer hen, non commercial layer hen, duck and Japanese quail.
3. To find out the prevalence of different serotypes of Salmonella in the contents and on the shell of different types of eggs
4. To find out the emergence of any specific Salmonella serotype as predominant egg associated salmonellae

2.4. Materials and Methods

2.4.1. Description of eggs

2.4.1.1. Commercial and non-commercial layer hen eggs

Commercial layer hen and non-commercial layer hen (maintained at the backyard of house hold) eggs have many uses in home, restaurants, and in institutions, either alone or as ingredients in other food stuffs. Shell eggs consist of
approximately 9.5% shell, 63% white, and 27.5% yolk. The shell is relatively porous, containing mostly calcium carbonate crystals; a keratin-type protein coats the shell and fills the pores. Two membranes separate the egg white from the shell. As the egg cools, an air cell forms generally at the large end of the egg where the two membranes separate. During storage, this air cell increases in size as water evaporates through the shell. A membrane also surrounds the yolk. The thick portion of the egg white, known as chalaza, holds the yolk near the centre of the egg.

Egg white contains approximately 10.5% to 11.5% solids, of which 86.0% is protein, 9.0% total reducing sugars (32% free glucose), and 5.0% ash. Only a trace of lipids is present. The pH of egg white is less than 8.0. Egg yolk contains 52% solids, of which 31.0% is protein, 64.0% is lipids, 2.0% is total carbohydrates, and 3.0% is ash. The pH of the yolk in freshly laid eggs is 6.0 and slowly increases to between 6.4 and 6.9. The pH of the blended yolk and white varies between pH 7.0 and pH 7.6.

2.4.1.2. Japanese quail egg

There are 45 species of quail. Although the Japanese quail is the largest species, it is much smaller than pigeon. While Indian quail weighs up to 100 gm and lays 100 eggs a year, the Japanese quail weighs up to 250 gm and lays 250 eggs a year. Japanese quails are raised for their tasty meat and nutritious eggs all over the world (Tikk and Tikk, 1993; Baumgartner, 1994; Yildirim and Yetisir, 1998).

Quail egg is roughly one-fifth the size of a chicken’s egg and weighs around 10 gm. The eggshells are spotted, with colors ranging from white to brown. Nutritionally, the quality of these eggs is at par with that of chicken eggs; rather they contain less cholesterol. The proportion of yolk (the yellow inside part) to
albumen (the white part), at 39:61, is higher compared to chicken eggs. They contain water 74%, protein 13%, fat 11%, carbohydrate 1%, total ash 1% and calorific value 649 Kj/100gm liquid. The mineral content includes 0.59 mg calcium, 220 mg phosphorus and 3.8 mg iron. The vitamin content comprises 300 mg of vitamin A, 0.12 mg of vitamin B1, 0.85 mg of vitamin B2 and 0.10 mg nicotinic acid.

2.4.1.3. Duck egg

Duck eggs are slightly larger than chicken eggs. They have a slightly higher fat content and somewhat more cholesterol than chicken eggs. Duck eggs also have more albumen (the protein in the white) than chicken eggs, which gives them more structure when cooked. The shells of duck eggs are thicker than those of chicken eggs and just a bit rubbery, which makes them harder to crack.

2.4.2. Sampling site

Eggs were collected randomly from different retail outlets near medical college, Kottayam. Total of 600 eggs (150 each for commercial layer hen, non-commercial layer hen, duck and Japanese quail (Kada) were analysed for the presence of Salmonella.

2.4.3. Collection and Transportation of Samples

Unwashed eggs were collected individually in sterile polythene bags and transported to the laboratory. Aseptic procedures were strictly adopted during collection. Samples were subjected to bacteriological examination within 1 hour after sampling.
2.4.4. Processing of samples

Swab technique was used to sample the egg surface of the intact eggs. The swab technique was selected because it is inexpensive and practical. Although its sensitivity and reproducibility are not optimal, it is considered to be satisfactory (O’Brien, 1988). Swabs were prepared in absorbent cotton and sterilized by autoclaving at 15 lb for 15 minutes.

For isolation of Salmonella, modified method of Hatha and Lakshmanaperumalsamy (1997) was used; the pre-enrichment medium lactose broth was replaced with buffered peptone water (Himedia, Bombay). The swabs were directly inoculated into 10ml buffered peptone water for pre-enrichment, in screw capped bottles and incubated at 37°C for 24 hrs.

In order to collect the egg content, eggs were washed thoroughly with soap and brush. Then surface sterilization was done by immersing the eggs in 70% ethyl alcohol for 30 min., air dried in sterile chamber for 10 minutes, then cracked with a sterile knife. Each egg content was mixed thoroughly and 25 ml of the mixed egg content was inoculated into 225 ml of buffered peptone water, and incubated at 37°C for 24 hrs. After 24 hours of incubation 1ml of the pre enriched broth was transferred into 10ml of tetra thionate broth (TTB) and selenite cystine broth (SCB) and incubated at 37°C for 24 hours for selective enrichment.

After 24 hours of selective enrichment a loopful of cultures from both SCB and TTB were streaked on to selective media such as xylose lysine deoxycholate (XLD) agar and hektoen enteric agar (HEA) plates and incubated at 37°C for 24 to 48 hours. Plates were observed after 24 hours incubation for typical Salmonella like colonies on XLD (pink colonies with black center) and HEA (greenish blue colonies with black centre), whenever present typical colonies were picked up; (few colonies
from positive plates) restreaked to ensure purity, and were maintained on TSA slants at room temperature for further characterization.

2.4.5. Primary biochemical screening

Purified colonies were subjected to primary biochemical screening involving reactions on triple sugar iron (TSI) agar, lysine iron agar (LIA) slants, indole production in tryptone broth and urease production on Christensen’s Urea agar. Cultures matching typical reaction of salmonellae (K/A, H₂S+ and gas + in TSI; K/K, H₂S+, gas ± in LIA; Urease and indole negative) were then subjected to secondary biochemical characterization.

2.4.5.1. Reaction on triple sugar iron (TSI) agar: In this test the ability of the organism to ferment three different sugars such as lactose, sucrose and dextrose were analysed. Suspected Salmonella cultures were inoculated into TSI agar by stabbing the butt and streaking the slant and incubated at 37°C for 24 hrs. The production of alkaline (red) slant and acid (yellow) butt indicated that the organism was able to ferment only glucose while unable to ferment lactose and sucrose. Blackening of the medium was considered as the production of H₂S, which is typical of Salmonella isolates.

2.4.5.2 Reaction on lysine iron (LIA) agar: In this test the ability of the organism to decarboxylate or deaminate lysine and form H₂S was evaluated. The Salmonella cultures were inoculated into the medium by stabbing the butt and streaking the slant and incubated at 37°C for 24 hrs. The production of alkaline reaction (purple colour) in slant and butt indicated the decarboxylation of lysine and blackening of the medium by the production of H₂S is considered positive for Salmonella.

2.4.5.3. Indole test: Indole test is used to check the release of indole ring from the breakdown of tryptophan by Salmonella. Presumptive Salmonella culture from nutrient agar were inoculated into tryptone broth and incubated at 37°C for 48 hours. After
incubation, 1ml of Kovac’s reagent was added to the medium. Development of a red ring in the reagent layer is considered as indole positive. Salmonellae were unable to breakdown tryptophan and release the indole ring and hence gave negative result.

2.4.5.4. Urease test: In this test the urea splitting ability of *Salmonella* culture by the production of enzyme urease was checked. Suspected cultures were inoculated into the Christensen urea agar by streaking the surface of the slant and incubated 37°C for 24 hrs. When urea is hydrolysed by the bacteria, ammonia accumulates in the medium and makes it alkaline. This increase in pH causes the indicator to change from dull yellow to deep pink colour and is considered as positive test for urease hydrolysis. As *Salmonella* does not utilize urea, failure to develop a deep pink color is considered as a negative test.

2.4.6. Secondary biochemical screening

In the secondary biochemical characterization, the presumptive cultures were tested for their ability to ferment various carbohydrates such as lactose, sucrose, dulcitol and salicin. Reactions matching typical biochemical reaction of *Salmonella* (lactose, sucrose, and salicin are negative and dulcitol positive) (Difco, USA) was further confirmed by slide agglutination test using polyvalent ‘O’serum against *Salmonella*.

2.4.7. Serotyping

The confirmed *Salmonella* cultures were sent to National *Salmonella* and *Escherichia* Centre (NSEC), Kasauli, Himachal Pradesh for serotyping of the strains.

2.4.8. Statistical analysis

The results were subjected to Two-Factor ANOVA without replication by using SPSS (Version: 11; SPSS Inc.1995).
2.5. Results

Salmonella contamination in commercial layer hen eggs, non-commercial layer hen eggs, duck and Japanese quail eggs collected from the retail outlets from Kottayam has been studied in the present investigation. The egg shell as well as the egg content was analysed. A total of 600 eggs (150 each for different types) were analysed for the presence of Salmonella.

2.5.1 Overall prevalence of Salmonella in the egg contents of different types of egg

Prevalence of Salmonella in the egg contents of different types of eggs shown in Table 2.1. The prevalence of Salmonella on egg content ranged from 1.33% in commercial layer hen to 51.33% in duck eggs.

Table 2.1. Prevalence of Salmonella in the egg contents of different types of eggs

<table>
<thead>
<tr>
<th>Type of egg</th>
<th>No. of samples analysed</th>
<th>No. of samples tested +ve for Salmonella</th>
<th>Percentage of incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial layer hen</td>
<td>150</td>
<td>2</td>
<td>1.33</td>
</tr>
<tr>
<td>Non-commercial layer hen</td>
<td>150</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Duck</td>
<td>150</td>
<td>77</td>
<td>51.33</td>
</tr>
<tr>
<td>Japanese Quail</td>
<td>150</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Chapter II

Prevalence of Salmonella in Eggs of Poultry, Duck, and Japanese Quail

Variation in the prevalence of *Salmonella* in the contents of different types of eggs were compared in Figure 2.1. Among the eggs, duck egg showed maximum prevalence of *Salmonella* in the egg content followed by eggs from non commercial layer hen. None of the egg contents of Japanese quail tested positive for *Salmonella*. The high degree of *Salmonella* contamination in the egg content of duck suggest transovarian route of infection.

![Figure 2.1](image)

Figure 2.1. Variation in the prevalence of *Salmonella* in contents of different types of eggs

2.5.2 Overall prevalence of *Salmonella* in the egg shell of different types of egg

Prevalence of *Salmonella* on the egg shell of different types of eggs shown in Table 2.2. Shell contamination with *Salmonella* was found to be relatively high on the eggs from commercial layer hens. Egg shell contamination with *Salmonella* was less than 10 percent in the eggs of house hold layer hen, Japanese quail and duck.
Table 2.2. Prevalence of *Salmonella* on the egg shell of different types of eggs

<table>
<thead>
<tr>
<th>Type of egg</th>
<th>No. of samples analysed</th>
<th>No. of samples tested +ve for <em>Salmonella</em></th>
<th>Percentage of Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial layer hen</td>
<td>150</td>
<td>31</td>
<td>20.66</td>
</tr>
<tr>
<td>Non-commercial layer hen</td>
<td>150</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Duck</td>
<td>150</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Japanese quail</td>
<td>150</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

Variation in prevalence of *Salmonella* on the shell of different types of egg is compared in Figure 2.2. While the shell surface had relatively higher levels of *Salmonella* contamination in case of the eggs from commercial layer hens, non-commercial layer hens and Japanese quail when compared with their egg contents. The prevalence of *Salmonella* on egg shell ranged from 4% to 20.66%. Commercial layer hen egg shell showed maximum prevalence of *Salmonella*. The *Salmonella* contamination in the commercial layer hen seem to be horizontal as most of the *Salmonella* positive samples were from egg shell, which is likely to be cross contamination from faeces or other samples from the cage. When compared with other types of eggs prevalence of *Salmonella* was very low in eggs from non-commercial layer hens.
2.5.3 Distribution of *Salmonella* serotype on different types of eggs

The confirmed *Salmonella* isolates were serotyped at National *Salmonella and Escherichia* Centre, Kasauli, Himachal Pradesh to find out the various serotypes. Results are presented in Table 2.3. *S.* Worthington, *S.* Weltevreden, *S.* Typhimurium, *S.* Bareilly, *S.* Dublin *S.* Enteritidis, *S.* Infantis are the serotypes encountered in different types of eggs studied. *S.* Worthington was the predominant serotype in the egg of commercial layer hen, while *S.* Bareilly was frequently isolated from the eggs of non commercial layer hen. *S.* Enteritidis and *S.* Infantis had nearly equal distribution in the eggs of duck. *S.* Bareilly was predominant serotype in the eggs of Japanese quail.
Table 2.3. *Salmonella* serotypes encountered in different types of eggs

<table>
<thead>
<tr>
<th>Type of egg</th>
<th>Salmonella serotype encountered</th>
<th>Antigenic pattern</th>
<th>Percentage of Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial layer hen</td>
<td>S. Worthington</td>
<td>1,13,23:z:1,w</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>S. Weltevreden</td>
<td>3,10:r:z6</td>
<td>4.16</td>
</tr>
<tr>
<td></td>
<td>S. Typhimurium</td>
<td>4,5:I:1,2</td>
<td>8.33</td>
</tr>
<tr>
<td></td>
<td>S. Bareilly</td>
<td>6,7:y:1,5:-</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>S. Dublin</td>
<td>9,12:gp:-</td>
<td>16.6</td>
</tr>
<tr>
<td>Non-commercial layer hen</td>
<td>S. Bareilly</td>
<td>6,7:y:1,5:-</td>
<td>77.7</td>
</tr>
<tr>
<td></td>
<td>S. Dublin</td>
<td>9,12:gp:-</td>
<td>22.2</td>
</tr>
<tr>
<td>Duck</td>
<td>S. Enteritidis</td>
<td>9,12,g,m:-</td>
<td>46.6</td>
</tr>
<tr>
<td></td>
<td>S. Infantis</td>
<td>6,7:r:1.5</td>
<td>53.3</td>
</tr>
<tr>
<td>Japanese quail</td>
<td>S. Worthington</td>
<td>1,13,23:z:1,w</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>S. Typhimurium</td>
<td>4,5:I:1,2</td>
<td>14.2</td>
</tr>
<tr>
<td></td>
<td>S. Bareilly</td>
<td>6,7:y:1,5:-</td>
<td>57.14</td>
</tr>
</tbody>
</table>
2.5.4 Relative prevalence of *Salmonella* in the egg content and on egg shell of different types of eggs

Figure 2.3 indicates the relative prevalence of *Salmonella* on the egg shell and contents of different types of eggs. In the case of duck eggs, egg content had considerably higher levels of contamination when compared with egg shell. The prevalence level was much lower in both shell and content of non-commercial layer hen eggs when compared to that of commercial layer hen egg. In case of Japanese quail eggs none of the egg content tested positive for *Salmonella*.

![Figure 2.3. Relative prevalence of *Salmonella* on the egg shell and in the contents of different types of eggs](image)

Statistical analysis of prevalence of *Salmonella* in the egg contents and on the egg shell of different types of eggs revealed that there was no significant difference (P>0.05) in the prevalence of *Salmonella* in the egg contents and on the egg shell of different types of egg.
2.5.5 Prevalence of *Salmonella* in the eggs of commercial layer hen

Percentage incidence of various *Salmonella* serotype in eggs from commercial layer hen was also carried out and the results are presented in Figure 2.4. Five different *Salmonella* serotypes were encountered in case of commercial layer hen eggs such as *S*. Worthington, *S*. Weltevreden, *S*. Typhimurium, *S*. Bareilly and *S*.Dublin. *S*. Worthington was the predominant serotype followed by *S*. Bareilly and *S*. Dublin.

Figure 2.4. Percentage incidence of various *Salmonella* serotypes in eggs from commercial layer hens

2.5.6 Prevalence of *Salmonella* in the eggs of non-commercial layer hen

Figure 2.5 indicate the percentage incidence of various *Salmonella* serotype in eggs from non-commercial layer hen. Two *Salmonella* serotypes were observed in case of non-commercial layer hen eggs such as *S*. Bareilly, *S*. Dublin. Isolation of *S*. Bareilly was more frequent when compared with that of *S*. Dublin.
Chapter II  
Prevalence of Salmonella in Eggs of Poultry, Duck, and Japanese Quail

2.5.7 Prevalence of *Salmonella* in the eggs of duck

Figure 2.6 indicate the Percentage incidence of various *Salmonella* serotype in eggs from duck eggs. Two *Salmonella* serotypes were encountered such as *S. Enteritidis*, *S. Infantis*. In the present investigation, *S. Enteritidis* was observed only in duck eggs.
2.5.8 Prevalence of *Salmonella* in the eggs of Japanese quail

The Percentage incidence of various *Salmonella* serotype in Japanese quail eggs are presented in Figure 2.7. There were three different serotypes such as *S.* Worthington, *S.* Typhimurium and *S.* Bareilly. *S.* Bareilly was the predominant serotype followed by *S.* Worthington.

![Bar chart showing the percentage incidence of *Salmonella* serotypes in Japanese quail eggs](chart.png)

Figure 2.7. Percentage incidence of *Salmonella* serotypes in the eggs of Japanese quail

Figure 2.8 indicates the relative incidences of various *Salmonella* serotypes in different types of eggs. Seven different serotypes such as *S.* Worthington, *S.* Weltevreden, *S.* Typhimurium, *S.* Bareilly, *S.* Dublin, *S.* Enteritidis, and *S.* Infantis were isolated from different types of eggs analysed in the present study.
Figure 2.8. Relative incidence of various *Salmonella* serotypes in different types of eggs

*S.* Enteritidis, *S.* Infantis were observed only in duck eggs. The presence of *S.* Dublin was observed both in commercial layer hen eggs and non-commercial layer hen eggs. *S.* Bareilly was isolated from the eggs of commercial layer hen, non-commercial layer hen and Japanese quail. *S.* Typhimurium was present in eggs of commercial layer hen and Japanese quail. The presence of *S.* Worthington was recorded both in the egg of commercial layer hen and Japanese quail but the presence of *S.* Weltevreden observed only in commercial layer hen eggs.
2.6. Discussion

Salmonellosis is still a major food borne disease in human. Modern practices in the poultry industry are even now very favourable to the maintenance and dissemination of Salmonella serotypes. The present investigation revealed the prevalence of Salmonella in various types of eggs such as eggs from commercial layer hens, non-commercial layer hens maintained at backyards, duck and Japanese quail. The phenomenon of worldwide increase in Salmonella is further supported by the results of the present study.

In the case of commercial layer hen eggs around 20% of egg shells were contaminated with Salmonella. The incidence levels of Salmonella in eggshell reported earlier were variable. In Spain, Perales and Audicana, (1989) reported around 1% Salmonella contamination. In the United Kingdom prevalence levels were reported to be varying from zero to 7% (Humphrey et al., 1989). The prevalence level in the present investigation is higher than these observations. But the prevalence of Salmonella is low in the commercial layer hen eggs when compared to the results of Peterson and James (1998). Higher prevalence of Salmonella on the shell of commercial layer hen eggs may be due to the poor cleanliness of the cage conditions. Since the shell contamination of egg is a case of horizontal transmission, prompt removal of poultry waste and disinfection can greatly reduce this. This is further strengthened by our observation that shell contamination in non-commercial layer hen was much lower. In this case the hens are maintained at the backyard at a much lower concentration. While it was negligible in the non-commercial layer hens grown on backyards and Japanese quail eggs.

The result clearly indicate that S. Worthington was the most frequently isolated serovar on the egg shell along with other serovars, such as S. Bareilly, S.
Typhimurium, and S. Dublin. There was no *Salmonella* contamination in the egg content of Japanese quail eggs.

The results of the present study highlights high degree of *Salmonella* contamination in the duck egg contents with *S. Enteritidis* and *S. Infantis*. *S. Enteritidis* is a serovar that can infect and cause disease in a broad range of hosts including poultry and number of mammalian species (Baumler et al., 2000). Currently, *S. Enteritidis* is the most common serotype reported in human cases of salmonellosis in the European Union and the second most common serotype (behind *S. Typhimurium*) reported in the United States (de Jong and Ekdahl, 2006; CDC, 2006). Like most *Salmonella* serotypes, *S. Enteritidis* has a variety of virulence factors that contribute to its pathogenicity.

In our results other serovars of *Salmonella* spp., isolated from the eggshells were not recovered in the egg contents. This suggests that not all the eggs with shell contamination had their contents contaminated. The contamination recorded in eggs content was only by *S. Enteritidis*, *S. Infantis*. This is indicative of trans-ovarian route of contamination by *S. Enteritidis* and *S. Infantis*. It is reported that the contamination of egg contents with *S. Enteritidis* is predominantly the result of infection of the reproductive tissue rather than passage through the shell after lay (Humphrey 1994). There was no association between shell contamination and the presence of *S. Enteritidis* of egg content laid by naturally infected hens (Mawer et al., 1989; Humphrey 1991).

However, the *Salmonella* contaminations in the egg contents of commercial and non-commercial layer hens were found to be negligible. *S. Enteritidis* associated salmonellosis has been reported as a pandemic involving several conditions in Europe and USA in the early 90’s (Rodriguez et al., 1993) and several initiatives such as Enternet and Pulsenet to track the presence of *S. Enteritidis* in eggs/poultry from
different parts of the world has come in place. Since food production in a globalised environment is mostly centralized and the chances of outbreak from a common source could likely arise. One of the objectives of the present investigation was to generate data on *Salmonella* contamination in egg for comparison with existing reports of *S.* Enteritidis in egg. The results clearly revealed that *S.* Enteritidis is yet to establish as a predominant serotype in hen’s eggs in our country.

However *S.* Enteritidis contaminations in duck eggs seem to be significant. Since the duck in our region are usually maintained in aquatic environments with considerable degree of microbial pollution, the possibility of accesses of pathogen such as *S.* Enteritidis to ducks is quite high. The results also indicate that *S.* Enteritidis is capable of doing transovarian contamination in ducks. However specific studies are required to prove this point.

It may be noted that an average consumer in India is not aware of the consequences of food poisoning and very often the producers and retailers are taking advantage of the situation. The eggs are not that well scrubbed and they reach fresh from production facility. This results in the presence of fecal matter on the eggshell and resultant presence of pathogen in case the hen is an infected one. Also the eggs are stored at room temperature, which again help the mesophilic microorganism such as *Salmonella* to multiply fast in case they get access to egg content through cracks which can develop during transportation and handling.