

SALMONELLA ENTERITIDIS – FOOD BORNE PATHOGEN – A REVIEW

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ABSTRACT

Salmonellosis has been a notifiable disease in the U.S since 1943, and S. enteritidis is the second most prevalent cause of infection after Salmonella typhimurium in humans. Salmonella is the leading cause of produced related outbreaks accounting for 30%. Because of the severity of this disease and apparent low infective dose (<10 cells) Salmonella is considered one of the most serious of known food borne pathogens. It is mainly pathogenic to human but in animals it does not induce any clinical symptoms except diarrhoea. So the animals act as carriers to Salmonella infection. The majority of transmission is through eating of undercooked eggs, chicken, raw milk, raw vegetables and fruits, contaminated water etc. The conventional isolation procedure includes preenrichment in Buffered peptone water for isolation of S. enteritidis at 37°C for 16 h to recover stressed cells and selective enrichment in Rappaportvassiliadis broth and tetrathionate broth at 42°C for 24 h. Since the infection primarily occurs via faeco-oral route, the preventive measures include proper cooking of meat and eggs, consumption of pasteurized milk, washing fruits and vegetables and drinking chlorinated water apart from personnel hygiene measures.

KEY WORDS

Food borne, Salmonella, Salmonella enteritidis.

INTRODUCTION

Salmonella enteric is one of the major bacterial agents that cause food borne infections in humans all over the world (1). Salmonellosis is a major economic problem for the food industry and a public health hazard in many countries (2). *Salmonella* infected samples must be quickly identified so that they can be isolated and spread of contamination can be controlled (3). *Salmonella* are among the major bacterial pathogens of poultry in the world (4). Prevention of *Salmonella* infection is important for poultry

health and for the food industry, and prevention can be achieved only by good monitoring and screening programmes (5).

In many countries human salmonellosis is mainly due to consumption of eggs followed by poultry, pork, beef, and dairy products (6). *Salmonella enteritidis* (*S. enteritidis*) has emerged as a pathogen of poultry in mid 1970's, but later became an important human pathogen. *S. enteritidis* is a major cause of human food borne illness and is the most frequent serovar detection in outbreaks of human salmonellosis (7). The increased consumption of fast food of

animal products and the international food trade between countries have also played an important role in spreading *S. enteritidis* (8). It can also spread via the environment through faecal contamination by humans and animals (9).

The number of *Salmonella*, to be swallowed in order to cause infection is rather small that is fewer than 10 (10) and it is a must for the livestock products to be tested for the presence of *Salmonella* due to its potentially low infective dose (11). The symptoms in human beings includes diarrhea, nausea, abdominal pain, mild fever, chills, vomiting, prostration, headache, malaise etc. and the diarrhea varies from thin vegetable soup like stools to a massive evacuation with accompanying dehydration (12).

Prevalence of *Salmonella enteritidis* and zoonotic importance

The first report of food poisoning linked to *Salmonella*, occurred in Germany in 1888, in which 50 persons, who consumed raw ground beef became ill and 1 died (13). Infection in poultry flocks, which is asymptomatic was first noticed in the late 1970's and in 1980's spread rapidly throughout UK, US, South America and other areas and its incidence increased substantially, showing a 275 fold increase in Argentina and becoming the predominant cause of this disease in U.S (14).

The incidence of *Salmonella typhimurium* infections declined and *S. enteritidis* grew from a serotype constituting only 6% of all *Salmonella* isolates in 1980 to become the most frequently reported *Salmonella* serotype isolated in U.S in 1990, constituting 21% of all *Salmonella* isolates that year (15). The incidence of *S. enteritidis* have dramatically increased from 5% (1976) to 26% (1994) among the salmonellosis cases reported to CDC (USDA, 1995) and in the last few decades, *S. enteritidis* has emerged as a major health problem worldwide (1).

Between 1975-1987 the Centers for Disease Control (CDC) determined that no. of *S. enteritidis* infections in North Eastern U.S had increased about 7 fold (17) and it has expanded in Europe and U.K. In 1990, *S. enteritidis* became the most frequently reported *Salmonella* serovars isolated in the U.S (18). Benouda *et al.* (19) reported that *S. enteritidis* represented 56.54% of the total isolated *Salmonella* strains in Morocco between 1990 and 1997; whereas it was 44% between 1994 and 2002 at Casablanca (20). *S. enteritidis* and *S. typhimurium* accounted for ~70% of all human and non human isolates of *Salmonella* reported worldwide between 1985 and 2008. Infact *S. enteritidis*, alone accounted for 61.4% of the 1.5 million human isolates of *Salmonella* reported during this period (21). From 2000 to 2002 a survey of global distribution of *Salmonella* serovars proved *Salmonella enteric sub spp enteric serovar enteritidis* to be the far most common serovar isolated from humans accounting for 65% of all isolates, more pronounced in Europe, where it is associated with 85% of all human cases (22). In Thailand the incidence of *S. enteritidis* among salmonellosis has increased in both human beings and animals in 2006 compared to 2005 (23).

Salmonella is still one of the most important food borne pathogen in industrialized countries with an estimated incidence of salmonellosis per 1,00,000 people ranging from 157 in 1999 to 92 in 2004, Belgium is one of the countries with the highest incidence compared with other European countries (24).

Pathogenesis

The virulence of *Salmonella* relates to their ability to invade host cells, replicate in them and resist both digestions by phagocytes and destruction by the complement components of plasma (25). Adhesion of *S. enteritidis* to cell surface by fimbriae is an essential stage in colonization and

pathogenesis of salmonellosis (26 & 27). The association and penetration of the bacterium in the digestive mucosa is a prerequisite for systemic infection (28). Following adherence, probably through fimbrial attachment to the surface of intestinal mucosal cells, the bacteria induce ruffling of cell membranes and releases effector proteins through type III secretion system (25).

In a susceptible host, *S. enteritidis* replicates primarily in the mucosa of digestive tract after oral challenge and localises in mucosa of ileum, caecum, colon and mesenteric lymph nodes and then spreads to the spleen, liver and various other organs and tissues (29). *S. enteritidis* colonizes the reproductive organs of infected birds without causing discernable illness and survives host defences during the formation of the egg (30). The production of a capsule like O antigen structure by certain wild type strains of *S. enteritidis* has been associated with reproductive tract tropism and improved survival within eggs (31). Grade A shell eggs have been attributed to the human *S. enteritidis* related illness because it can infect ovarian tissues of laying hens and be deposited into the developing egg, resulting in contamination of egg contents (32).

Transmission

Salmonella can be introduced into poultry houses by means of contaminated feeds, particularly those that contain animal raw materials (33) or by water or vectors such as insects, rodents, persons and equipment (34). Wild rodents and wild life are known as vector for *Salmonella*, flies may be involved in the dissemination of *Salmonella* in the environment as carrier of microorganisms because flies were positive for *Salmonella* on the highly contaminated farms (35). The transmission of *S. enteritidis* to humans by internally contaminated table eggs possesses a challenge to

public health, even though efforts to control this problem are at higher side (36).

S. enteritidis only rose to its predominant position in man and poultry in the mid 1980's as a result of vertical and horizontal transmission within and between large poultry organizations in many parts of the world (37). Grade A shell eggs have been attributed to the human *S. enteritidis* related illness because *S. enteritidis* can infect ovarian tissues of laying hens and be deposited into the developing egg, resulting in contamination of egg contents (38&32). Ingestion is the main route of infection in Salmonellosis although, it can occur through the mucosa of the upper respiratory tract and conjunctiva (39).

Human salmonellosis

Salmonellosis has been a notifiable disease in the U.S since 1943, and *S. enteritidis* is the second most prevalent cause of infection after *Salmonella typhimurium* in humans (21). *S. enteritidis* emerged as an important pathogen in poultry and human (27). Human *S. enteritidis* infections showed a dramatic increase since 1980's, particularly in developing countries and has become the most commonly isolated serotype in many countries (40). In Canada, *S. enteritidis* has been among the top 3 reported *Salmonella* serovars resulting in human disease since 1998, accounting for between 12-21% of infections caused by *Salmonella* (41). *S. enteritidis* emerged as a major cause of human salmonellosis worldwide throughout the 1980's-1990's and out of 309 *S. enteritidis* outbreaks with a confirmed source, 214 (78%) were associated with raw or undercooked shell eggs between 1990-2001 (36). *S. enteritidis* is clinically prevalent serovar associated with the consumption of foods containing eggs or poultry meat from systemically infected chicken (42) and it is the most common serovar in poultry and humans in Finland (43).

S. enteritidis infects fowls such as chicken without severe illness, but when transmitted to humans,

it causes severe diarrhoea and bacteraemia (44). The infection dose of non-typhoidal *Salmonella* for humans generally described as 10^2 to 10^3 organisms; however doses of 10 to 20 organisms and even fewer than 10 organisms have caused illness (10). Forshell and Wierup (12) reported clinical signs include diarrhoea, nausea, abdominal pain, mild fever and chills. The diarrhoea varies from a few thin vegetable soup like stools to a massive evacuation with accompanying dehydration. Vomiting, prostration, anorexia, headache and malaise may also occur with syndrome lasts for 2 - 7 d. Gantois *et. al.* (30) reported that *S.enteritidis*, upon infection of a human host causes self limiting gastroenteritis, similar to that caused by other non typhoidal *Salmonella* serovars. Invasive complications like osteomyelitis, pyoarthrosis, nephrosis (45), lung abscess, appendicitis, cholecystitis, salphingitis, peritonitis, pneumonia, pleurisy, cerebral abscess (46), urinary tract infection (47), endocarditis (48), empyaema, purulent meningitis, intestinal perforation (49), hepatitis (50) and haemolytic ureamic syndrome (51) have been reported. Average fatality rate in *Salmonella* infections have been reported to be 5.3% (45). But certain serotypes can cause a fatality rate of upto 21% (52).

Food borne salmonellosis

Salmonella is the most common food poisoning bacteria associated with refrigerated poultry (53). As all *Salmonella* serovars are primarily pathogenic for animals, the most common source of *Salmonella* infection for humans is contaminated food of animal origin (54). *Salmonella enterica* serovar *enteritidis* is the main cause of food borne salmonellosis (55) and it has been a major causative agent of food borne gastroenteritis in humans (56). The majority transmission is through eating of undercooked contaminated chicken, meat and consumption of raw eggs, egg products, salads, vegetables, fruits,

chocolate, maize snack and also through bean sprouts (57&58). Hence the prevalence and occurrence of *S.enteritidis* in foods of animal origin are detailed here under.

Eggs

More than 70% of *S.enteritidis* infections reported, the source of infection has been traced to grade A table eggs (38). Eggs are contaminated internally with *S.enteritidis* probably as a result of infection in the hens ovaries and oviducts (59). Egg contamination can originate before oviposition by direct contamination of the yolk, albumen, or egg shell membranes with bacteria from the infected reproductive organs of the birds or after or during the oviposition by penetration of bacteria from contaminated faeces through the egg shell (30). Hogue *et. al.* (60) reported that 10% to 40% of pasteurized liquid eggs at commercial egg breaking plants contain *S.enteritidis* in USA.

Salmonella enterica serotype *enteritidis* has been associated with food borne outbreaks in which contaminated egg-based foods such as homemade icecream, cookie batter, caesar salad and hollandaise sauce, were consumed (61). *S.enteritidis* emerged as a major cause of human salmonellosis worldwide throughout the 1980's-1990's and out of 309 *S.enteritidis* outbreaks with a confirmed source, 214 (78%) were associated with raw or undercooked shell eggs between 1990-2001 (36). Infected hens can deposit *S.enteritidis* into the edible contents of developing eggs which poses a continuous problem for controlling salmonellosis (62 & 63). Eggs cooled had 91-100% penetration by this organism compared to uncooled eggs which had 48% penetration (64).

Poultry Meat

Boonmar *et. al.* (65) reported that some *Salmonella* infections in humans are due to the consumption of contaminated broiler chicken meat. Hoop and Pospischil (66) tested 7 samples

taken from ovary and oviduct of poultry which are positive by culture method, 6 were shown positive for *S. enteritidis* by immune histochemical method. Recently it has been identified that poultry, consumption of raw eggs, poultry meat, processed products, fast food and international food trade between the countries are the major sources of infection and transmission of *S. enteritidis* in humans (67). Wang *et. al.* (68) tested 285 samples of chicken meat, eggs and swabs of chicken related samples and reported that only 1% of the samples were positive for *Salmonella enteric serovar enteritidis*. Hassanien *et. al.* (69) examined 75 samples of beef, chicken and reported that *Salmonella* was detected in 20% of minced frozen beef, 36% of frozen chicken leg and 52% of the frozen chicken fillets and the prominent *Salmonella* serovars were *S. enteritidis* and *Salmonella kenchuki*.

Milk

Salmonella organisms has been isolated from milk and its products. Most milk borne salmonellosis has been associated with raw, heat treated, or inadequately pasteurized milk or with milk contaminated after pasteurization (70). Ryan *et. al.* (71) reported a large outbreak of salmonellosis in U.S with an estimated number of 1,80,000 cases caused by the consumption of 2 brands of pasteurized milk produced at the same dairy. Bean *et. al.* (72) reported an outbreak of salmonellosis involving 1,50,000 persons in the U.S attributed to pasteurized milk. Milk products like cheese have also been implicated in a number of salmonellosis outbreaks (73). Post-processed skimmed milk (74), fresh cream cakes (75) and spray dried milk powder (76) have also been reported to cause salmonellosis.

Pork and Beef

Zaidi *et. al.* (77) studied the incidence of non-typhoidal *Salmonella* in pork and beef from retail shops in Mexico and reported that out of 339 and 126 pork and beef samples tested 197 (58.1%)

and 68 (54%) were positive for *Salmonella* organisms. *Salmonella* organisms has been isolated from beef (78), chevon (79), mutton (80). Several outbreaks of *Salmonella* food poisoning associated with eating beef have also been reported (81).

Other Foods

Human salmonellosis has also been associated with fresh fruits, vegetables and vegetable salads (82) and fresh fruit juices (80). Foods commonly associated with outbreaks of *Salmonella* are milk based products (chocolate, cheese, salads, ice cream etc.), sliced cold meat, vegetables and fruits (57). A wide variety of foods are however known to be associated with *S. enteritidis* including fresh and dry fruits and vegetables, where contamination can occur during fertilization, irrigation, harvesting or handling under non-optimal hygiene conditions (32). Evans *et. al.* (83) and Arumugaswamy *et. al.* (84) reported salmonellosis outbreaks consequent to the consumption of fish and shell fish. In a study conducted by Wilson and Moore (85), *Salmonella* serotypes were recovered from 8% of 433 mollusks examined.

Faeces and environmental samples

Poultry faeces

Prescott and Gellner (86) reported that 18% of chicken flocks examined contained *S. enteritidis* in the chicken's colon at the time of slaughter. *S. enteritidis* may be transmitted between the poultry via faecal contamination and when *S. enteritidis* was orally infected, was found in the birds livers, caeca, oviducts and ovaries after 3 wk (87). Turkyilmaz *et. al.* (88) used PCR method for isolation and identification of *S. enteritidis* and reported that out of 587 cloacal swabs collected from turkeys 86 (14.7%) were *Salmonella* positive and out of 86 *Salmonella* 11 (1.9%) were *S. enteritidis*.

He *et. al.* (89) estimated the no. of *S. enteritidis* after oral challenge in different organs of poultry and reported that *S. enteritidis* reached a peak at 24 h - 36 h post inoculation, with the liver and spleen containing high concentrations of *S. enteritidis*, whereas the blood, heart, kidney, pancreas, gall bladder had low concentrations. *S. enteritidis* populations began to decrease and were not detectable at 3 d post inoculation, but were still present upto 12 d post inoculation in the gall bladder, 2 wk for liver and 3 wk for spleen without causing apparent symptoms.

Human faeces

The number of *Salmonella* organisms present in the faeces of an infected human beings will be approximately 10^9 /g, which will fall gradually (90) and 5% of the patients upon recovery from this disease may become carriers who shed the organisms in their faeces (91). Hassanien *et. al.* (69) examined 28 children stool cultures collected from hospitalized children with diarrhoea, fever and reported that 10.71% (21) were positive for *Salmonella*.

Other birds faeces

Vanhoosar and Welsh (92) isolated *Salmonella* from ratites and reported that 46 out of 248 ostrich samples and 34 out of 99 emu samples and 16 out of 60 rheas were positive for various spp. of *Salmonella*. Turkyilmaz *et. al.* (88) isolated *Salmonella enteric sub. spp. enteric serovar enteritidis*, a cause of food poisoning in humans from turkeys and reported that of the 587 cloacal swabs, 86(14.7%) were *Salmonella* positive and of these, 11(1.9%) were *serovar enteritidis*.

Environmental Samples

Gast *et. al.* (63) used electrostatic air sampling device for collection of air samples 3 times a week from experimentally infected laying hens for isolation of *S. enteritidis* and reported that electrostatic collection of air was superior over traditional methods for detecting *S. enteritidis* in environmental samples.

DIAGNOSIS

The detection of *Salmonella* in foods is problematic due to presence of fewer no. of organisms, together with larger no. of competing microflora and also due to injured organisms by different food processing methods (93). The conventional culture method, which is routinely used for isolation of *Salmonella* is time consuming, laborious and may not be suitable for viable but non culturable (VBNC) state of the organisms (11). PCR is rapid, specific and sensitive method for the detection of food borne pathogens (94). The increase in the frequency of the outbreaks and the no. of cases has prompted public health officials for accurate detection within few hours.

The conventional isolation procedure involves pre enrichment in Buffered peptone water (BPW) for isolation of *S. enteritidis* at 37°C for 16h to recover stressed cells (95) and selective enrichment in Rappaportvassilidias broth and tetrathionate broth at 42°C for 24h. The most commonly used selective enrichment for isolation and identification of *S. enteritidis* are Rapaport Vassilidias soya broth, Selenite cystine broth and Tetrathionate broth (3). Then streaked on selective agar like BGA, XLD, HEA, BSA at 37°C for 24h. *Salmonella* will give black centered colonies.

PREVENTION AND CONTROL

Since the infection primarily occurs via faeco-oral route, the preventive measures include food hygiene measures like proper cooking of meat and eggs, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine treated water and personnel hygiene measures like washing hands after toilet visits.

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