

## A Review on the Public Health Importance of Bovine Salmonellosis

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### Abstract

Salmonellosis is an infectious disease of human and animal caused by organisms of the two species of *Salmonella* (*S. enterica* and *S. bongori*). A *Salmonella* pathogen are highly adaptive and potentially pathogenic to human and other domestic animals; young, pregnant and lactating animals are the most susceptible and are capable of producing a serious of infections having foodborne zoonosis importance. Enteric disease is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicaemia, abortion, arthritis and respiratory disease, may be seen. Worldwide there are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths. Poultry, egg, meat, dairy products and fruits and vegetables serve as vehicles of transmission. Most human salmonellosis cases are foodborne, but each year infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoological gardens, farm environments or other public, professional or private settings. *Salmonella* passes through M-cells overlying Peyer's patches, through dendritic cells or through the epithelial lining in the lower part of small intestine or proximal colon to arrive in the sub epithelial location which is also transported to extra intestinal sites such as the liver, spleen and mesenteric lymph nodes. *Salmonella* pathogens are common in cattle and potentially infect human that come in contact with cattle. Bovine salmonellosis is caused by *S. typhimurium* and *dublin*. The disease in cattle is characterized by septicemia, acute or chronic enteritis or abortion. *Salmonella enterica* sub species *enterica* develop a resistance to multi antibiotics in which results in increasing failure of treatment and severity of infection. Basic hygiene practices and the implementation of scientific based management strategies can efficiently mitigate the risks associated with animal contacts. However, the general public is frequently unaware of the specific disease risks involved and high-risk behaviors are common. The disease can be also controlled by vaccination of cattle.

**Keywords:** Cattle; Foodborne; Human; *Salmonella*; Zoonosis

### Introduction

The genus *Salmonella* was named after Daniel E. Salmon who first reported the isolation of *Salmonella* from a pig in 1885 and named the organism *Bacterium choleraesuis* (currently known as *Salmonella enterica* serovar *Choleraesuis*) [1,2]. *Salmonella* causes gastroenteritis and typhoid fever and is one of the major foodborne pathogens of significant public health concern [3]. *Salmonella* is considered as one of the most wide spread foodborne zoonosis in industrialized as well as developing countries even though the incidences seems to vary [4]. Salmonellosis is a disease caused by many serotypes of *Salmonella* and characterized clinically by one or more of the three major syndromes; septicemia, acute and chronic enteritis [5].

*Salmonellae* are common in cattle. They are often concern due to disease of cattle and the potential to infect human that come in contact with cattle or consume dairy product or bovine meat product. Meat processing and packaging at the whole sale or retail level contribute to higher levels of contamination in minced beef product compared to beef carcass. The presences of even small number of *Salmonella* in carcass meat and edible offal may lead to heavy contamination of minced meat. When meat is cut in to pieces more microorganisms are added and increased surface area of exposed tissue from the contaminated equipment. Raw meat particularly, minced meat has a very high total count of microorganisms of which *Salmonella* are likely to present large number [6].

Bovine salmonelosis usually manifest clinically as a syndrome of septicemia, acute or chronic enteritis and abortion [7]. There are few serotypes that are associated with cattle and of this *Salmonella enterica* subspecies *enterica* serotype *Dublin* (*S. dublin*) and *Salmonella enterica* subspecies *enterica* serotype *Typhimurium* (*S. typhimurium*) is the most common [8]. The presence of *S. typhimurium* in cattle and the cross contamination of beef carcass tissue is one of the most common cause of *Salmonella* infection in developed countries [9].

In addition to causing infection many *Salmonella* isolates particularly *S. typhimurium* definitive type (DT) 104 have developed resistance to multiple antibiotics. Many isolate of this phage type are resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (ACSUT) with increasing number of isolate showing resistance to trimethoprim and fluoroquinolones [10,11].

The resistance organism may act as donor of resistant determinant to another facultative pathogen of the human commensal flora of intestinal tract which may later be associated with disease and, in turn, supply the resistances gene to other pathogen [12].

There are more than 2500 different *Salmonella* serotypes and all are considered potentially pathogenic to human [13]. In human *Salmonella* is one of the most common causes of bacterial gastroenteritis. Death from *Salmonella* food poisoning estimated 1,000 deaths each year in USA [14]. The economic loss associated in human salmonellosis is due to investigation, treatment and prevention of illness [15]. Therefore, the objective of this review paper is to show the effect of eating of raw or undercooked meat, drinking of unpasteurized milk from the perspective of public health and to forward recommendation based on conclusion.

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Received March 29, 2014; Accepted May 26, 2014; Published May 28, 2014

Citation: Kemal J (2014) A Review on the Public Health Importance of Bovine Salmonellosis. J Veterinar Sci Technol 5: 175. doi:10.4172/2157-7579.1000175

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## General Overview of Bovine Salmonellosis

### Classification

The current nomenclature of the genus *Salmonella* adopted worldwide through different publications is the one used by CDC is based on the recommendations from the WHO collaborating centre and it adequately addresses the concern and requirements of clinical and public health microbiologists (Table 1) [16]. Scientifically *Salmonella* classification is described under:

Domain: Bacteria

Phylum: Protobacteria

Class: Gamma Protobacteria

Order: Enterobacteriales

Family: *Enterobacteriaceae*

Genus: *Salmonella*, and

Species: *Salmonella enterica* and *Salmonella bongori* [17]. The species *Salmonella enterica* being divided into six subspecies (I–VI); *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV) and *S. enterica* subsp. *indica* (VI) [18] on the basis of biochemical characteristics (biotype), differences observed in multilocus enzyme electrophoresis (MLEE), phylogenetic analysis using 16S rRNA or other sequences, or analyses using other molecular techniques such as amplified-fragment length polymorphism (AFLP). Members of *Salmonella enterica* subspecies I account for 99% of all human infections [19].

### Etiology and characteristics

Strains of genus *Salmonella* obey the definition of the family *Enterobacteriaceae*, they are straight rod usually motile with peritrichous flagella (except *S. pullorum* and *S. gallinarum*), facultative anaerobe, ferment glucose usually with production of gas (except *S. typhi* and *S. dublin*), but failed to ferment lactose, sucrose, salicin and urea, reduce nitrate to nitrite and most are phototropic [20,21]. *Salmonella* multiply optimally at a temperature of 35°C to 37°C, pH about 6.5-7.5 and water activity between 0.94-0.84. They are chemo-organotrophic organisms [22]. They are also able to multiply in the environment with low level or no oxygen [23]. The bacteria are sensitive to heat and will not survive a temperature above 70°C; so it is sensitive to pasteurization, but resist to drying even for years. Especially in dried feces, dust and other dry materials such as feed and certain food [24].

<i>Salmonella</i> species and subspecies	No. of serovars within subspecies	Usual habitat
<i>S. enterica</i>	2557	
<i>S.e. ssp. enterica</i>	1531	Warm blooded animal
<i>S.e. ssp. salamae</i>	505	Cold blooded animals and environment
<i>S.e. ssp. arizonae</i>	99	Cold blooded animals and environment
<i>S.e. ssp. diarizonae</i>	336	Cold blooded animals and environment
<i>S.e. ssp. houtenae</i>	73	Cold blooded animals and environment
<i>S.e. ssp. Indica</i>	13	Cold blooded animals and environment
<i>S. bongori</i>	22	Cold blooded animals and environment
<b>Total (genus <i>Salmonella</i>)</b>	<b>2579</b>	

Table 1: Currently recognized number of *Salmonella* serovars [62].

Species	Serovars	Common syndrome
Cattle	<i>S. dublin</i> and <i>S. typhimurium</i>	Septicemia, acute and chronic enteritis and abortion
Sheep	<i>S. typhimurium</i>	Septicemia, typhocolitis and abortion
Pig	<i>S. choleraesuis</i> <i>S. typhimurium</i> , <i>S. typhisuis</i>	Septicemia
Horses	<i>S. typhimurium</i>	Septicemia, acute colitis and abortion
Chicken	<i>S. pullorum</i> , <i>S. gallinarum</i>	Septicemia, acute and chronic enteritis
Human	<i>S. enteritidis</i> , <i>S. typhi</i> , <i>S. paratyphi</i>	Gastroenteritis, bacteremia

Table 2: The most common serovars of *Salmonella* that infect livestock and syndrome they induce [63].

*Salmonella* strains are serologically classified using Kauffmann-White scheme, and at the present the genus contains more than 2500 serotypes [13,25,26]. However, a report from the Centre for Infectious Disease Research and Policy (CIDRAP) classifies members of the *Salmonella* species into more than 2541 serotypes (serovars) according to their somatic lipopolysaccharide (O), flagellar (H) antigens and at times capsular (Vi) antigen and most strains show diphasic variation of the flagellar antigens [27,28]. Recently, Diagnostic Services of Manitoba (DSM) suggested the number of *Salmonella* serotypes that causes disease in human now approaches 3000 and new serotypes are identified continuously [29] (Table 2).

### Epidemiology

The epidemiology of *Salmonella* is complex which often makes control of disease difficult. Epidemiological pattern of prevalence of infection and incidences of disease differ greatly between geographical area depending on climate, population density, land use farming practice, food harvesting and processing technologies and consumer habits. In addition, the biology of serovar differs so widely that *Salmonella* infection or *Salmonella* contamination are inevitably complex [24].

**Geographical distribution:** *S. enteritidis* is the most prevalent species followed by *S. typhimurium* which are a worldwide distribution. Change in the relative frequency of serotypes can be observed over a short period of time. Some times within one or two years only limited number of serotypes is isolated from man or animals in a single region or country and the predominance of one or other can vary over a time. Some serotypes like *S. enteritidis* and *S. typhimurium* are found worldwide in contrast to *S. weltevreden* which seems to be confined to Asia [30]. Sibhat et al. [31] found the serovars Newport, Anatum and Eastbourne to be the most prevalent in Ethiopia.

**Occurrences:** The rate of infection in domestic animal has been estimated from 1-3%. In 1980, 16274 strains of 183 serotypes of *Salmonella* were isolated in USA from samples of meat obtained from slaughter house. In other examination of animals positive culture was obtained from 4.59% of 141, 827 bovine fecal samples. Epidemiological surveillance of animals including bird is of the most important since source of large majority of non-typhoidal salmonellosis cases are of food animal origin. There is scarce of data from developing countries in this regard [30].

**Morbidity and mortality:** In a case control study of *S. typhimurium* DT 104 infection in cattle in Great Britain, Evans and Davies [32] reported an overall case fatality rate of 44%. In the herd with outbreak of *S. typhimurium* DT 104 the case fatality rate was 51.2% in calves, 37.4% in adult cattle and 26.2% in fattening cattle [32]. In dairy sucker and

mixed herd the case fatality rate was found 44% where in calf rearing unit and dealer herd was found 50%-100% [33]. However modern antibiotic therapy may have reduced this rate. In an outbreak of *S. Dublin* in calf rearing unit 29 (13.5%) of 214 calves died [34]. Mortality was significantly higher in group reared calves (19.25%) than in calves in single pens (9.2%). Mortality and morbidity is usually highest in calves under 12 week of age. In all species case fatality rate often reach 100% if treatment is not provided [24].

**Economic importance:** Salmonellosis is a significant cause of economic loss in farm animals because of the cost of clinical disease, which include death, diagnosis and treatment of clinical cases, diagnostic laboratory cost, the cost of cleaning and disinfection and cost of control and prevention. In addition when the disease is diagnosed in the herd, it can create a considerable apprehension in the producer because of difficulty on identifying infected animals. An estimation of economic impact of an outbreak of *S. Dublin* infection in calf rearing unit indicate that the cost of disease represented a substantial proportion of gross margin of rearing calves [24]. Estimated annual costs for salmonellosis have ranged from billions of dollars in United States to hundreds to millions of dollars in Canada and millions of pounds in United Kingdom. Analysis of five *Salmonella* outbreak due to manufactured food in North America gave direct cost with range from \$36,400-\$62 million, there have been few studies in to the cost and benefit of preventing *Salmonella* infection, but it has been suggested that for every £1 spent on investigation and curtailment of the outbreak there is a saving of £5 [35].

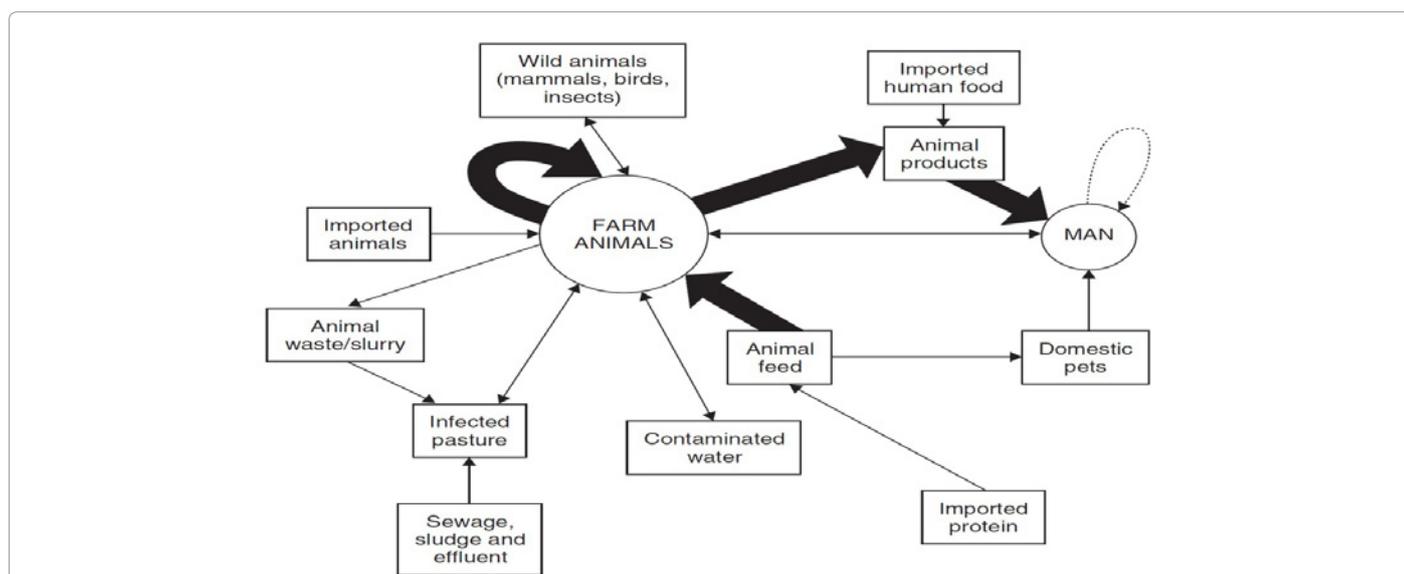
**Source of infection and transmission :** Most *Salmonella* infection in farm animals are likely to acquire from animals of the same species, especially in the case of the host adapted serovars. In adult cattle there are important differences in the behavior of *S. dublin* and *S. typhimurium*. Those animals which recover from clinical *S. dublin* infection may become persistent excretors, shedding up to 10<sup>6</sup> organisms per gram of faeces daily. Other herd may harbor infection and excrete the organisms only when stressed particularly at parturition. Aerosol transmission has long been suggested as a means by which *Salmonella* may be transmitted and experimental infection

of calves by aerosol has been reported recently. In addition pasture contamination results when flooding occurs and there are many reports of clinical case in adult cattle arising from grazing recently flooded pasture [35] (Figure 1).

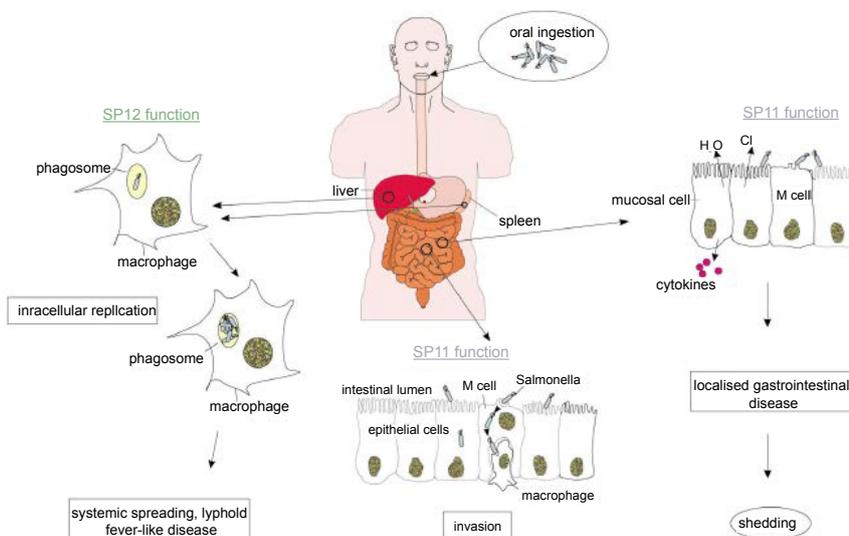
### Pathogenesis

The outcome of infection with *Salmonella* depends essentially on three factors: the infective dose, predisposing factor influencing the host and the level of immunity [7]. In cattle the common route of infection is ingestion of the bacteria through contaminated feed and water. It may disseminate to the rest body part of the host via the lymph fluid or blood and usually also lead to faecal excretion of bacteria. Salmonellae are normally inhibited by the high concentrations of volatile fatty acids and the normal pH below 7 in the rumen [36]. These bacteria have developed mechanisms to survive and cope with the host inhibiting factors, but the normal inhibition of *Salmonella* is primarily disrupted in the rumen and small intestine when (i) starvation or reduced feed intake occur [37], (ii) the feeding strategy leads to an increased pH in the abomasum, and (iii) antibiotic treatment kills the normal competing microflora of the intestine [38]. The bacteria adhere to and invade intestinal cells in the mucosa mainly associated with the Peyer's patches in the terminal jejunum and ileum through the columnar enterocytes and specialized microfold enterocytes (M cells). Once the bacteria have crossed the intestinal epithelium they enter macrophages in the underlying lymphoid tissue from where they are drained to the local lymph nodes, which are important barriers for further dissemination. If this barrier is overcome, the bacteria reach the reticuloendothelial tissue containing organs while surviving and replicating inside the macrophages [39].

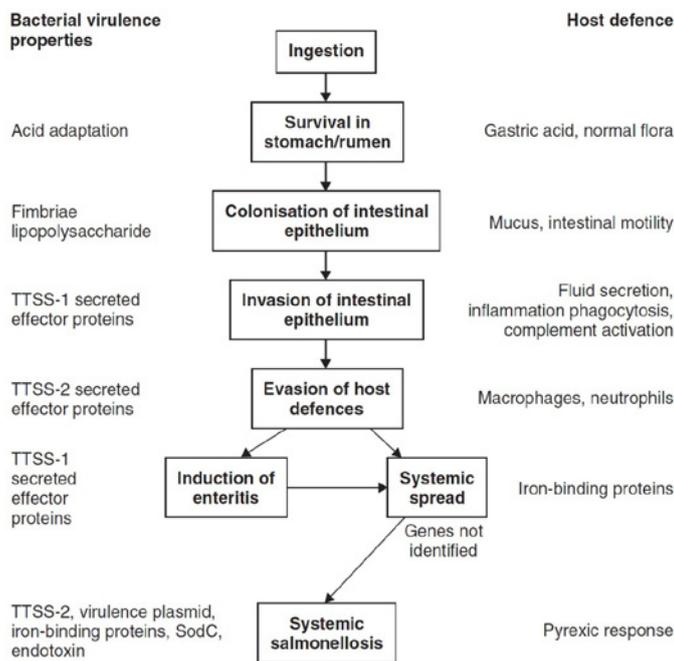
The virulence of *Salmonella* is related to their ability to invade host cell and resist both digestion by phagocytes and destruction by complement system [40]. The specific O antigen is important for the virulence strain of *Salmonella* (specific antigen) by decreasing the susceptibility of phagocytosis and ability to activate the alternative complement pathway [41]. Three toxins namely endotoxin, enterotoxin and cytotoxin play an important role in the pathogenesis of *Salmonella*. The endotoxin produce fever, the enterotoxin produce



**Figure 1:** Figurative representation of *Salmonella* spp. indicating source of infection and transmission [53]. Feed, water, pasture, wastes; wild animals etc can serve for the transmission of the pathogen *Salmonella* into farm animals which in turn serve as a source for human.



**Figure 2:** Schematic representation of host-pathogen interactions during pathogenesis of *Salmonella* infections in human. For the first stages of salmonellosis, *Salmonella* Pathogenicity Island (SPI1) function is required, i.e. the entry of *Salmonella* into nonphagocytic cells by triggering invasion and the penetration of the gastrointestinal epithelium and also for the onset of diarrhoeal symptoms. The function of SPI2 is required for later stages of the infection, i.e. systemic spread and the colonization of host organs [43].



**Figure 3:** The attachment, colonization, invasion, virulence property and host defence mechanism of *Salmonella* once ingested from food and pathogenicity island function [53].

mucosal damage in the cell culture and cytotoxin inhibit protein synthesis [42] (Figure 2).

In human the bacteria travel to the stomach and pass to the intestine, where they invade the cells, causing diarrhoea to their infected host body. *Salmonella* Pathogenicity Island (SPI1) function is required for the initial stages of salmonellosis, i.e. the entry of *Salmonella* into

nonphagocytic cells by triggering invasion and the penetration of the gastrointestinal epithelium. Furthermore, SPI1 function is required for the onset of diarrheal symptoms during localized gastrointestinal infections. The function of SPI2 is required for later stages of the infection, i.e. systemic spread and the colonization of host organs. The role of SPI2 for survival and replication in host phagocytes appears to be essential for this phase of pathogenesis [43] (Figures 2 and 3).

**Post mortem findings:** There may be no gross lesion in animals that have died peracutely but extensive submucosal and subserosal petechial haemorrhage are usually evident [24]. In adult cattle, a typical case reveals acute muco/necrotic enteritis, especially of the ileum and large intestine. The wall is thickened and covered with yellow-grey necrotic material overlying a red, granular surface. The mesenteric lymph nodes and spleen may be enlarged. In calves, the small intestine typically shows a diffuse mucoid or mucohaemorrhagic enteritis and the mesenteric lymph nodes are oedematous, congested and greatly enlarged [44].

**Pathological findings :** In adult cattle acute haemorrhagic enteritis with necrotic change usually most pronounced in the mucosa of large intestine is commonly found at postmortem examination and similar change may be seen in the small intestine. The mesenteric lymph node are in variably swollen, edematous and in many case haemorrhagic. The liver may show degenerative change and the wall of gall bladder may be thickened. In calves, the clinical sign are those of septicemia which resemble those described for adult cattle i.e. congestion and haemorrhagic areas in the intestine, enlarged lymph node and congested spleen. Petechial haemorrhages may be seen in the cortex of the kidney and epicardium [35].

The chronic form is manifested by discrete areas of necrosis of the wall of the cecum and colon. The wall is thickened and covered with yellow gray necrotic material overlying a red granular mucosal surfaces. *Salmonella* are present in the heart, blood, spleen, liver, bile, mesenteric lymph node and intestinal contents in both septicemic and acute forms. In the chronic form the bacteria may be isolated from the intestinal lesions and less commonly from other viscera [24].

### Clinical Sign

Adult cattle generally contract either acute or subacute enteric salmonellosis and pregnant animal may abort during the early stage of acute enteric disease. Severely affected animals show fever, depression, inappetence and drop in milk yield. These sign are followed by diarrhea which is foul smelling, the faeces being mucoid and usually containing a clot of blood and shred of necrotic intestinal mucosa. Sign of colonic congestion of mucus membrane and dehydration may be evident. The acute disease last for about a week. *S. dublin* in particular but also other serovars may cause abortion in cows at any stage of pregnancy (Table 3). Abortion may either precede the onset of dysentery or follow it within two or four weeks. Alternatively abortion may occur in cows that show no sign of illness, septicemia and/or placentitis being the cause of death of fetus. Retention of the placenta occurs in approximately 70% of cases that abort but subsequent fertility is not usually affected [7].

In calves clinical disease is most common 2-6 week of age. Clinical sign vary but typically the enteric form of diseases predominate which is characterized by pyrexia, dullness and anorexia, followed by diarrhea that may contain fibrin and mucus. The faeces may become blood stained and "stringy" due to the presences of necrotic intestinal mucosa. Calves rapidly become weak and dehydrated unless treated. Infected

calves usually die 5-7 days after the onset of disease. At this stage the organisms has become systemic as a result of reduced innate immunity and may be isolated from variety of tissue including blood. Calves that recover from infection do not typically remain carrier. Salmonellosis is very variable and in some animals particularly in very young animals rapid multiplication occur both in intestine and systemically associated with poor absorption of specific immunoglobulin G from colostrums or with calves receiving in sufficient or no colostrums [45].

### Disease in Human

#### Distribution

Worldwide there are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths due to *Salmonella*. In the US annually there are 2-4 million cases with a death rate of 500-1,000 and an economic loss of about 3 billion dollars. A recent CDC report indicates that the incidence of *S. typhimurium* decreased significantly (42% decline) from 1996-1998 to 2005; however, the incidence of other serotypes are on the rise such as *S. enteritidis* and *S. heidelberg*, each of which increased by 25% and *S. enterica* serovar Javiana increased by 82% [46].

#### Physiology

The *Salmonella*, in the family *Enterobacteriaceae*, are facultatively anaerobic, gram negative and rod-shaped bacteria. Given the close relationship with *E. coli* and the many years devoted to its study, the overall metabolism of *Salmonella* is well understood. The cells are typically 0.7-1.5 µm by 2-5 µm. They grow at 7-48°C with an optimum growth at 37°C (mesophile) and at pH 4.05-9.5 with an optimal growth at pH 6.5-7.5 (neutrophile). *Salmonella* grows optimally at a water activity of 0.995 and are chemo-organotrophs. They have both fermentative and oxidative metabolism. The primary route for metabolism of carbohydrates is the Embden-Meyerhof pathway (glycolysis). They ferment glucose to formate (with the production of gas) and to ethanol, acetate, or lactate [19].

#### Transmission to human

Human salmonellosis is generally foodborne and is contracted through consumption of contaminated food of animal origin such as meat, milk, poultry and eggs. Dairy products including cheese and ice cream were also implicated in the outbreak. However, fruits and vegetables such as lettuce, tomatoes, cilantro, alfalfa-sprouts and almonds have also been implicated in recent out-break [35].

Acute gastroenteritis is usually acquired from consumption of food which may be directly or indirectly contaminated with *Salmonella*. A wide variety of animal species have been shown to be capable of harboring the organisms and in the developed world turkey, chicken, swine and cattle are found to be infected carriers in the studies conducted in the abattoirs. These carriers may readily shed *Salmonella* during transportation to the abattoir and contaminate abattoir workers or equipment during slaughter. The progressive trend forwards mass

<b>S. dublin</b>	<b>S. typhimurium</b>
Bovine specific usually endemic herd problem	Not host-specific usually sporadic disease outbreaks
Bovine carriers important as a source of infection	Various animal species and their product are important sources of infection
Carriers excrete organism for long times or sometimes (long life)	Carrier excrete organism for short period usually 3-4 months
Septicemia is common	Septicemia is uncommon: enteritis is common
Abortion relatively common	Abortion uncommon

**Table 3:** Important epidemiological and clinical difference between *S. dublin* and *S. typhimurium* infection in cattle [7].

processing and distribution of food products has been an important factor in the increase incidences of *Salmonella* foodborne diseases. Person to person spread has been demonstrated on many occasions and may take place in young children and group living under poor socioeconomic condition where effective sanitation is lacking. Person to person spread also may occur in hospitals, nursing homes, mental institution in which large number of outbreak has occurred. Amplification of infection in these institutions may occur from contaminated food or asymptomatic carrier's babies being at special risk [35]. Direct or indirect contact with animals colonized with *Salmonella* is another source of infection, including contact during visits to petting zoos and farms [47].

Fecal oral route and vehicle born infection may result from ingestion of food or water that have been contaminated with human or animal feces or from direct exposure to animals or their waste. A lower infectious dose of organism is usually required in the elderly, the immunocompromised, antibiotic users and those with achlorhydria or regular use of antacid and related medication. The commonly recognized vehicle of transmission includes inadequate cooked or raw meat, unpasteurized milk or milk product, contaminated and inadequately treated drinking water. www.dol.gov.

Contamination of milk may occur by a variety of route. Animal may occasionally, excrete the organisms in milk during the febrile stage of the disease or more likely infected feces, from either a clinically infected cow or healthy carrier may contaminate the milk during the milking process. Milk also may be contaminated from use of polluted water from dirty equipment or from dairy workers. Indirect contamination also has been described when cattle have become contaminated with *Salmonella*. Contamination of food also may occur directly from *Salmonella* infected food handlers or indirectly from sewage polluted water [48].

### Clinical Sign in Human

The incubation period depends on the number of bacteria ingested and varies from 5-72 hours. Affected individual's experience sudden nausea, vomiting and watery foul smelling diarrhea which is in most case last only a few hours. If the colon is affected, the stool may also contain blood and or mucus. Fever up to 39°C is not uncommon. Convalescences within 1-2 days but the illness may last for 5-7 days [49].

More severe symptom may occur in people who are at high risk like those extreme age groups (the young because their immune system are immature and the elderly because the immune system are declining), person with decreased gastric acidity (because gastric acid is the first line of defense for the ingested *Salmonella*), person with altered gastric intestinal bacteria (including those taking broad spectrum antibiotics, purgatives or who have had bowel surgery) and person taking opiate drug in which the bowel movement is decreased. In these highest risk groups of people *Salmonella* may invade beyond the gastrointestinal tract (GIT) to cause severe systematic illness.

*Salmonella* gastroenteritis is usually a self-limiting illness and fatality is uncommon. Biopsy and endoscopic examination have demonstrated the colon to be a major site of infection. The change in the colon ranges from edema of lamina propria with a focal or diffuse inflammatory infiltrate to a more intensive inflammation with disruption of the surface epithelium and multi-focal micro abscesses. In more severe cases, vascular congestion, infiltration of the lamina propria with polymorphonuclear leucocytes and abscess formation has been recorded [35].

### Public Health Aspect of Bovine Salmonellosis

Salmonellosis is an important global public health problem causing substantial morbidity and thus also has a significant economic impact. Although most infections cause mild to moderate self-limited disease, serious infections leading to deaths do occur [50]. In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, foodborne diseases still dominate as the most important public health problem in most countries [51].

A salmonellosis incidence is defined as the identification of *Salmonella* from animals or group of animal's product or surrounding which can be specifically related to identifiable animals or from animals feed. On the human side, a registered medical practitioner in the US required under the Public Health (Control of Disease) act to notify the local authority, if the patient is suffering from or suspected of having foodborne disease [35].

Studies provide increasing evidence of adverse human health consequences due to the occurrence of resistant microorganisms. Use of antimicrobial agents in human and animal affects the intestinal tract placing those concerned at increased risk of certain infection. This is defined as the proportion of *Salmonella* that would not have occurred if the *Salmonella* were not resistant. In addition antimicrobial agent used in animal can result in increased transmission of resistant microorganisms between animal and therefore would results in case of transmission of such microorganisms to human through food. Increased frequency of treatment failure and increase severity of infection may be manifested by prolonged duration of illness. www.who.int/mediacenter.

*Salmonella dublin* is largely but not entirely specific to cattle with average 10 human case reported in each year in Ireland. Apart from its pathogenicity two other characteristics of *S. dublin* make it particularly important for Ireland from a public health viewpoints. First, it is very prevalent on Irish farms and secondly in evolutionary terms, it is only one step away from *S. enteritidis*, a common *Salmonella* serotype in poultry and the main case of clinical salmonellosis in humans. In genetic terms, difference between the serovars *S. dublin* and *S. enteritidis* are no greater than those found within each serotypes. This indicates that, *S. dublin* and *S. enteritidis* share a common ancestor. One branch evolved in to a poultry adapted serotype capable of causing disease in human, the other in to host specific cattle pathogen. If *S. dublin* has been confirmed in breeding herd there is a significant risk of persistent infection in carrier cows for as long as animal which were present at the time of the outbreak remain in the herd [53].

### Diagnosis of Salmonellosis

The clinical sign and finding at postmortem examination are not unique to salmonellosis although a tentative diagnosis may be made. They should confirm either in diseased animal or at necropsy by isolation of organisms in their feces and determination of viable counts. Fecal samples rather than swabs should be taken and these should obviously be obtained before administration of antibiotics. It may be also possible to isolate organism from oral secretion and by blood culture although these are less reliable than feces culture and must be taken with care to avoid contamination. Animal that died of salmonellosis usually have large number of *Salmonella* distributed throughout their tissue and sample of spleen, liver, hepatic, mediastinal and bronchial lymph nodes may yield count in excretion of 10<sup>6</sup> organisms/gram. Similar concentration may be present in the wall and content of the ileum, cecum, colon and associated lymph nodes. Sample should be taken from internal organs in order to distinguish animal that have died of enteritis without septicemia [53].

## Culture Methods

The traditional *Salmonella* culture method involves pre-enrichment, selective enrichment, isolation of pure culture, biochemical screening and serological confirmation, which requires 5-7 days to complete. The USDA and FDA recommended method involves a 6-24 h pre-enrichment step in a nonselective broth such as lactose broth, tryptic soy broth, nutrient broth, skim milk, or buffered peptone water with a recommended incubation temperature of 37°C. The selective enrichment step requires additional 24 hours incubation in Rappaport-Vassiliadis (RV) broth, selenite cystine (SC) broth, or Muller Kauffmann tetrathionate broth. The inoculation temperature of 41.5°C ± 1°C for RV broth and 37°C ± 1°C for SC and MKTT broth is used. Bacterial cells are isolated from selective agar plates such as Hektoen enteric agar (HEA), xylose lysine deoxy-cholate (XLD), and/or brilliant green agar (BGA). Biochemical testing is done using triple sugar iron agar and lysine iron agar, which requires an additional 4–24 hours [54,55].

## Rapid detection methods

**Detection of antibodies to salmonella by enzyme immunoassay (EIA):** The detection of antibodies to *Salmonella* by EIA offers a sensitive and cost-effective method for mass screening of animal flocks/herds for indications of a past/present *Salmonella* infection. The limitation of the method is that the immune response of the individual animal is not elicited before 1-2 weeks after infection takes place. A number of commercial kits are available for testing poultry, cattle and pigs. An obvious advantage of this method is that it can be automated and no incubation is required to increase the numbers of bacterial cells [56].

The EIA is a well-established technique for assaying antigens. Antibodies labeled with an enzyme are bound to *Salmonella* antigens, and the level of antigen present is determined by enzymatic conversion of a substrate, usually resulting in a color change which can be read visually or by a spectrophotometer. The EIAs rely on the standard cultural procedures for pre-enrichment and selective enrichment to provide enough *Salmonella* cells for detection. EIA technology that enables detection at an earlier stage of resuscitation and/or culture can provide even more rapid results. Serological test, such as ELISA, serum agglutination and complement fixation can be used for the retrospective diagnosis of salmonellosis or the detection of carriers [55].

**Nucleic acid-based assays:** Real-time quantitative polymerase chain reaction using PCR (Q-PCR), reverse transcriptase PCR (RT-PCR), and nucleic acid sequence-based amplification (NASBA) have been used for detection of *Salmonella* from various food matrices. *Salmonella enterica* was detected at 1 cfu ml<sup>-1</sup> after a culture enrichment of 8-12 h in the TaqMan-based Q-PCR using *invA* gene as target. NASBA method has been used for detection of viable *Salmonella* cells and it has been demonstrated to be more sensitive than RT-PCR, and moreover, it requires fewer amplification cycles than the conventional PCR methods [46].

## Differential diagnosis

The differential diagnosis of *Salmonella enterocolitis* in humans include viral gastroenteritis and bacterial enteric infections including those caused by *Shigella*, Enterotoxigenic *E-coli*, *Campylobacter* species, *Vibrio* species and *Yersinia enterocolitica*. The disorder must also be differentiated from toxin-mediated foodborne illness including those caused by *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens*. Compared to the patient with toxin mediated disorder person with salmonellosis usually have less prominent vomiting and are the more likely have prolonged diarrhea and fever. The shorter

incubation period of toxin mediated foodborne illness can be important to distinguish them from salmonellosis. A recent history of ingestion of raw milk, undercooked meat and contact with farm animals 48 hours before the onset of illness suggests the diagnosis of salmonellosis [49].

## Treatment

Treatment of non-typhoidal *Salmonella* infection is different from typhoidal infection. In treatment of non-typhoidal *Salmonella* infection antibiotics should not be used routinely, as used in typhoid. Antibiotic should be only used if required as most infection with non-typhoidal *Salmonella* is self-limiting type and duration of diarrhea and fever are not much affected by use of antibiotics. Additionally antibiotic therapy can increase relapse of infection and also prolong the duration of gastrointestinal carrier states. The main treatment should be aimed at correcting dehydration that may arise due to prolonged diarrhea by fluid and electrolyte replacement. [www.webcan.gogleuserconten.com](http://www.webcan.gogleuserconten.com).

Although salmonellae are usually sensitive *in vitro* to many antibiotics, their use for treatment of uncomplicated gastroenteritis until recently has been generally contraindicated by their lack of favorable effect on the course of the disease and by prolongation of *Salmonella* shedding. Following the introduction of fluoroquinolones a number of clinicians have advocated their use for treatment of only on enteric fever but also of *Salmonella* gastroenteritis because of their efficacy in reducing the duration of illness and of *Salmonella* shedding. In case of patient with bacteremia and other complication antimicrobials are used. Likewise the treatment of enteric fever necessitates the use of antimicrobial drugs with chloramphenicol, ampicillin, amoxicillin, trimethoprim-sulfamethoxazole and newer fluoroquinolones being drug of choice against sensitive *Salmonella*. Proper management of fluid and electrolyte balance is important in all patients with *Salmonella* gastroenteritis but is crucial in young children and elder individuals [35].

In animal treatment supportive treatment with intravenous fluid is necessary for patients that have anorexia, depression, significant dehydration. Individual patient may be treated aggressively following acid base and electrolyte assessment. Oral fluid and electrolyte may be somewhat helpful and much cheaper than IV fluid for cattle demand to be mildly or moderately dehydrated. The effectiveness of oral fluid may be somewhat compromised by malabsorption and maldigestion in salmonellosis patient but still should be considered useful. Cattle that are willing to drink can have specific electrolyte (NaCl, KCl) added to drinking water to help correcting electrolyte [57].

The implementation of broad prophylactic strategies that are efficacious for all salmonellae may be required in order to overcome the diversity of *Salmonella* serovars present on farms, and the potential for different serovars to possess different virulence factors [58]. Early treatment is essential for septicemic salmonellosis but there is controversy regarding the use of antimicrobial agent for intestinal salmonellosis. Oral antibiotic may alter the intestinal micro flora and interfere with competitive antagonism and prolong shading of the organism. There is also a concern that antibiotic resistance strain of *Salmonella* selected by oral antibiotic may subsequently infect human. Antibiotic such as ampicillin or cephalosporin lead to lyses of bacteria with release of endotoxin. NSAID may be used to reduce the effect of endotoxemia [5].

## Prevention and Control

### Prevention and control in animals

Condition that contribute to an increasing incidence of epidemic

salmonellosis include large herd size, more intensive and crowded husbandry and the trend of free-stall barn with loose housing, which contribute to the fecal contamination of the entire premise. When salmonellosis has been confirmed in a herd, the following control measure should be considered; isolate obviously affected animals to one group if possible, treat severely affected animals, affected animals institute measure to minimize public health concern like (no raw milk should be consumed) physically clean the environment and disinfect the premise following resolution of the outbreak or crises period. A mastitis survey should be conducted that include bulk tank surveillance. Prevention is best accompanied by maintaining a cross herd and culturing new feed additives and components before using the entire ration [57].

It is generally agreed that supportive therapy and good nursing are important. These include oral or parenteral re-hydration, correction of electrolyte balance and stabilization of acid base equilibrium [7].

**Salmonella vaccinology:** Calves vaccinated at 1-3 weeks of age with a modified aromatic dependant *S. dublin* bacteria have detectable anti-lipopolisaccharide immunoglobins after immunization. Safe live oral vaccine against *S. typhimurium* and *S. dublin* has been constructed and shown to control protection against experimental infection with virulent wide type strain of the organisms. A virulent *S. choleraesuis* vaccine is efficacies experimentally against salmonellosis due to *S. dublin* in calves to protect young calves. The best program is to vaccinate the cow during pregnancy which will give passive protection to calves for 6 weeks [24].

Generally, both live and attenuated vaccines produced from rough strain in bacteria commercially. There is some evidence that inactivated bacterins can induce a lower level of protection [59]. The veterinarian research institute produced vaccine against bovine salmonellosis in activated bacteria prepared from isolate of *S. dublin*, *S. typhimurium* and *S. bovismorbificans* and a live attenuated vaccine containing a virulent rough mutant of *S. dublin* [7].

### Prevention and control in human

There is no vaccine to prevent salmonellosis in human whereas, vaccine against *Salmonella typhi* has been developed, especially in children, but is only 60% effective. A person given this vaccine would still have a strong chance of developing salmonellosis. It is not expected that there will be a single vaccine that is effective against all the different forms of *Salmonella* soon. Ongoing research is investigating what can be done to produce a useful human vaccine for *Salmonella* [59]. People should not eat raw or uncooked meat, they should not drink raw milk or unpasteurized dairy product, cross contamination of food should be avoided. Uncooked meat should be kept separate from cooked food ready to eat. Hands, cutting boards or knives and other utensils should be washed thoroughly after handling uncooked food. Hands should be washed before handling any food and in between handling different food items. People should have to wash their hands after contact with animal's feces [46].

Pasteurization of milk and treating municipals water supply for reducing risk of *Salmonella* infection, improvement in farm animal hygiene in slaughter process in food harvesting and in packaging operation have helped to prevent salmonellosis [55]. A periodic surveillance of the level of *Salmonella* contamination in different food product and environment is necessary to control spread of the pathogen. Reducing *Salmonella* prevalence requires comprehensive control strategy in animal and animal foodstuff with restriction in

the infected flock until they have been cleaned from infection. In addition mandatory testing before slaughter should be conducted like the one implemented in Sweden [60]. Ensuring safe food production requires knowledge on the nature and origin of animal, animal feed, the health status of animals at the farm, the use of veterinary medicinal data regarding antimortem and postmortem findings and the risk association with post harvests production strategies [61].

### Conclusion and Recommendation

*Salmonella* is a leading cause of foodborne disease in human and consumption of both meat and milk has been implicated in salmonellosis outbreaks of people. Having animals and raw products it is not possible to be free from zoonotic agent; however the occurrences can be minimized by applying high standard of hygiene in all steps of the food production. Infected animals can present with a great variety of clinical symptoms, and risk factors for transmission to humans clearly differ by animal species, age groups, animal purpose and geographic region. In addition, strains of *Salmonella* resistant to multiple antibiotics have been isolated from dairy cow during salmonellosis outbreak on dairy operation. The same strains have also been isolated from ill people. A high degree of interaction between medical and veterinarian surveillance is needed. Finally, implementing basic and applied research to the agent that cause foodborne salmonellosis will be a crucial point for new approaches to prevent and control the disease.

### Based on the above facts the following recommendations are forwarded:

- Strict hygiene of the slaughter house and lairage
- People should not drink unpasteurized milk or milk products and should not eat raw meat.
- Establishment of HACCP
- Education of food handlers
- Vaccination of cattle
- Maintenance of cold chains
- Setting import standards
- Sanitary examination of the product
- Collaboration between government agencies, professional organizations and special interest groups.
- New legislations or public awareness campaigns have leads to dramatic decreases in *Salmonella* incidence.

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