

Review Article

Salmonella and Its Status in Ethiopia

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Salmonellosis is caused by *salmonella* organism, which is a gram negative, flagellated, facultative anaerobic, rod shaped, flagellated bacterium belongs to family of Enterobacteriaceae. *Salmonella* is one of the major and important foodborne pathogens of humans and animals causing salmonellosis, which have great medical and economical cost. *Salmonella* has significant public health implications causing food borne and zoonotic diseases in humans. Foodborne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs. *Salmonella* can be transmitted to humans along the farm-to-fork continuum, commonly through contaminated foods of animal origin, namely poultry and poultry-related products (eggs), pork, fish etc. For *Salmonella* to colonize its hosts through invading, attaching, and bypassing the host's intestinal defense mechanisms such as the gastric acid, many virulence markers and determinants have been demonstrated to play crucial role in its pathogenesis; and these factors included flagella, capsule, plasmids, adhesion systems, and type 3 secretion systems. Studies indicated the widespread occurrence and distribution of *Salmonella* in Ethiopia. In Ethiopia, the habit of raw meat consumption and the presence of *Salmonella* in minced beef indicate, in addition to the poor hygienic standards in food handling in the country, the presence of great public health hazards of *Salmonella*.

Keywords: Ethiopia; *Salmonella*; Virulence factors**Abbreviations**

BGA: Brilliant Green Agar; EIA: Enzyme Immunoassay; ELISA: Enzyme Linked Immunosorbent Assay; HEA: Hektoen Enteric Agar; Hr: Hour; iNTS: Invasive Non-Typhoidal Salmonellosis; LPS: Lipo Polysaccharides; Ml: Milliliter; NASBA: Nucleic Acid Sequence Based Amplification; PCR: Polymerase Chain Reaction; RV: Rappaport Vassiliadis; SC: Selenite Cystine Broth; SPIs: *Salmonella* Pathogenicity Islands; TSI: Triple Sugar Iron; TTSS: Type III Secretion System; WHO: World Health Organization; XLD: Xylose Lysine Desoxycholate

Introduction

In human beings, *salmonella* is among the most common causes of bacterial gastroenteritis worldwide, and food animals are important reservoirs of the bacteria [1]. Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteremia and enteric fever [2]. The genus *Salmonella* is a gram negative, rod shaped bacteria facultative anaerobe flagellated bacterium belongs to family of Enterobacteriaceae [3]. It is the most frequently isolated foodborne pathogen, and is predominantly found in poultry, eggs and dairy products [4]. Additional food sources that are involved in the transmission of *Salmonella* include fresh fruits and vegetables [5]. The disease is a fatal, spread to human being from eating of improperly cooked and raw foods such as meat, dairy products, eggs, unpasteurized milk, and bakery products and by direct contact with faeces/diarrhoea from infected animals [6].

Bacterial virulence factors have a crucial role for systemic

infections [7]. The virulence of *Salmonella* spp. is associated with a combination of chromosomal and plasmid factors [8]. Virulence factors are encoded by a number of genes located on the bacterium own chromosome, the so-called housekeeping genes, which give specific and basic characteristics to bacteria from the same family. These genes can be found in the so-called pathogenicity islands, or in mobile genetic elements such as transposons, plasmids and bacteriophages. These genes confer advantages for bacteria such as resistance to antimicrobials, adaptation to the host cell and the ability to overcome host defense mechanisms [9].

The ability of *Salmonella* strains to persist in the host cell is crucial for pathogenesis, as strains lacking this ability are non-virulent [10]. The clinical characteristics of salmonellosis in large animals vary depending on the various management systems used, the intensity of stocking, whether or not the animals are housed, and the epidemiological characteristics of the different *Salmonella* species [11]. The disease is most satisfactorily described as three syndromes, classified arbitrarily according to severity as septicemia, acute enteritis, and chronic enteritis. Studies indicated the widespread occurrence and distribution of *Salmonella* in Ethiopia. In Ethiopia, minced beef is usually used for the preparation of a popular traditional Ethiopian dish known as locally "Kitfo" and most of the time it is consumed raw or medium cooked. The habit of raw meat consumption and the presence of *Salmonella* in minced beef indicate, in addition to the poor hygienic standards in food handling in the country, the presence of great public health hazards of *Salmonella* [12].

Therefore, the main objective of the study is to review

salmonellosis and its status in Ethiopia.

Etiology

Salmonellosis is caused by *salmonella* organism, which is a gram negative, flagellated, facultative anaerobic, rod shaped, flagellated bacterium belongs to family of Enterobacteriaceae possessing three major antigens: H or flagellar antigen, O or somatic antigen VI antigen or superficial antigen overlaying the O antigen it is present in a few serovars [13].

Virulence Factors of *Salmonella*

Virulence plasmids

Not all isolates of these serotypes carry the virulence plasmid, but least six serotypes of *Salmonella* (serotypes Abortusovis, Choleraesuis, Dublin, Enteritidis, Gallinarum/Pullorum, and Typhimurium) are known to harbor a virulence plasmid. All plasmids contain the 7.8 kb *salmonella* plasmid virulence (spv) locus. This locus harbors five genes designated spv RABCD. Expression of the spv genes might play a role in the multiplication of intracellular *Salmonellae* [14]. The product of the spvR is a positive regulatory protein essential for the expression of the other spv genes [15]. The role of these plasmids in virulence has not been clarified yet [14].

Toxins

Both of endotoxins and exotoxins can be produced by *Salmonella*. Endotoxin is the lipid portion (lipid A) of the outer membrane lipopolysaccharide (LPS) of *Salmonella*, elicits a diversity of biological responses both in vivo and in vitro [16]. The exotoxins can be subdivided in two types: the enterotoxins and the cytotoxins. Cytotoxins are defined as by their ability to kill mammalian cells. There is a significant difference in the amounts of toxin produced by either serotype [17].

Fimbriae

Fimbriae are diverse proteinaceous surface structures. Fimbriae are a major player in pathogenesis and a source of diversity for *Salmonella* serovars. Fimbriae are the most common adhesion systems and are differentially expressed and found in a specific pattern among each serovar [18]. The fibres are implicated in attachment to surface. The name fimbria (Latin word for fibres) was suggested in 1955 to describe the filamentous structures [19]. The term fimbria is preferable to use to describe non-flagellar filaments than pili, which is used to designate structures implicated in conjugation [20]. Most of the *Salmonella* serovars possess 12 fimbrial gene clusters. Some fimbriae are specific to certain serovars and may play a role in these bacteria that do not need to be fulfilled in other serovars. A specific fimbrial gene cluster (FGC) encodes for the structural, assembly and sometime regulatory proteins required for the production of the filamentous adhesive appendage on the bacterial surface. However, fimbriae are implicated during infection and in a variety of other roles, like biofilm formation, seroconversion, haemagglutination, cellular invasion and macrophage interactions [21]. Fimbriae are important determinants of host adaptation by *Salmonella* [22].

Flagella

The bacterial flagellum is a long filamentous organelle responsible for motility. *Salmonella* swims in liquid environments and moves on solid surfaces by rotating flagella. In addition, the flagella also

facilitate bacterial adhesion and biofilm formation. Toll-like receptor 5 recognizes flagellin to activate the host immune system. Thus, the flagellum is also a considerable target to detect bacterial pathogens. The filament works as a helical propeller to propel the cell body [23].

Type III secretion systems

Central to the pathogenesis of *S. enterica* is the function of specialized protein secretion systems, known as Type III secretion system (TTSS). TTSS are specialized virulence devices that have evolved indirect translocation of bacterial virulence proteins into the host cell cytoplasm. Type III secretion systems are composed of several proteins that form a remarkable needle-like organelle in the bacterial envelope [24]. So far the presence of two SPIs (SPI-1 and SPI-2) each encoding a TTSS, have been described for *Salmonella* species and may reflect the flexibility of this highly successful pathogen in causing different forms of diseases [25].

Pathogenesis

The ability of *Salmonella* strains to persist in the host cell is crucial for pathogenesis, as strains lacking this ability are non-virulent [10]. Following the engulfment of *Salmonella* into the host cell, the bacterium is encased in a membrane compartment called a vacuole, which is composed of the host cell membrane. Under normal circumstances, the presence of the bacterial foreign body would activate the host cell immune response, resulting in the fusion of the lysosomes and the secretion of digesting enzymes to degrade the intracellular bacteria. However, *Salmonella* uses the type III secretion system to inject other effector proteins into the vacuole, causing the alteration of the compartment structure. The remodeled vacuole blocks the fusion of the lysosomes and this permits the intracellular survival and replication of the bacteria within the host cells. The capability of the bacteria to survive within macrophages allows them to be carried in the reticuloendothelial system (RES) [26].

Epidemiology

The epidemiology of salmonellosis is complex, which often makes control of the disease difficult. The epidemiological patterns of prevalence of infection and incidence of disease differ greatly between geographical areas depending on climate, population density, land use, farming practices, food harvesting and processing technologies, and consumer habits. In addition, the biology of the serovars differs so widely that considerations of salmonellosis, *Salmonella* infections or *Salmonella* contamination are inevitably complex [11].

Risk Factors Predisposing to Clinical Disease

The clinical characteristics of salmonellosis in large animals vary depending on the various management systems used, the intensity of stocking, whether or not the animals are housed, and the epidemiological characteristics of the different *Salmonella* species [11].

Animal risk factors

The response to infection with a *Salmonella* sp. varies depending on the size of the challenge dose and the immunological status of the animal, itself dependent on colostrum intake in neonates, previous exposure to infection and exposure to stressors, particularly in older animals [11].

Environmental and management risk Factors

Intensification of husbandry in all species is recognized as a factor contributing significantly to an increase in the new infection rate. Any significant change in management of the herd or a group of animals can precipitate the onset of clinical salmonellosis if the infection preexists in those animals. Temperature and wetness are most important, as *salmonellas* are susceptible to drying and sunlight [11].

Pathogen risk factors

Salmonellas are facultative intracellular organisms that survive in the phagolysosome of macrophages and can therefore evade the bactericidal effect of antibody. Compared to other organisms of the same family, *salmonellas* are relatively resistant to various environmental factors. They multiply at temperatures between 8°C and 45°C, at water activities above 0.94, and in a pH range of 4-8. They are also able to multiply in an environment with a low level of or no oxygen [11].

Human Source: The environmental and personal hygiene is one of the knowledge and practice restrictions of human from beef/dairy farm and abattoir food processing plants. On the other hand food getting contamination depends largely on the health status of the food handlers [27]. Food borne diseases are a public health problem in developed and developing countries like Ethiopia, the contamination occurs at any point during its journey through production, processing, distribution, and preparation [28]. High standards of hygiene of personnel are required to maintain in food processing industries and dairy farms [29].

Others Source: International trading and its introduction through international travel, human migration, food, animal feed and livestock trade are also other challenges; Water source: *Salmonellae* can be found in contaminated water; Inanimate objects. Moreover, In recent years, antimicrobial resistance of *Salmonella* has increased worldwide, due to the widespread use of antimicrobial drugs in the human and veterinary sectors, is the other ambiguities in the food processing environment [28].

Mode of Transmission

Salmonellas are spread by direct or indirect means. Infected animals are the source of the organisms; they excrete them and infect other animals, directly or indirectly by contamination of the environment, primarily feed and water supplies. The farm animal may be infected in different ways: by animal-to-animal transmission, especially of host-adapted serovars; by contaminated animal feed; and by a contaminated environment (soil, birds, rodents, insects, water supplies). The excretion of *salmonellas* is exacerbated by the stress imposed [11].

Transmission of *Salmonella* to humans traditionally has been attributed to contaminated animal-product foods, but epidemiological studies have demonstrated that cases are sporadic and may more likely involve environmental sources than previously thought. It has been suggested that contaminated soils, sediments and water as well as wildlife may play a significant role in *Salmonella* transmission [30]. Consumption of raw milk, inadequately pasteurized milk, improperly cooked beef from culled dairy cattle, contaminated water and direct animal contact are the major routes of acquiring dairy associated

salmonellosis in humans [31].

Status of *Salmonella* in Ethiopia from 2003-2017

Food borne diseases are public health problems both in developed and developing countries. Thousands of millions of people fall ill and may die as a result of eating unsafe food [32]. Biological contaminants largely bacteria, constitute the major cause of food borne diseases [33]. *Salmonella* infection most commonly occurs in countries with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking [34].

Studies indicated the widespread occurrence and distribution of *Salmonella* in Ethiopia. In Ethiopia, minced beef is usually used for the preparation of a popular traditional Ethiopian dish known as locally "Kitfo" and most of the time it is consumed raw or medium cooked. The habit of raw meat consumption and the presence of *Salmonella* in minced beef indicate, in addition to the poor hygienic standards in food handling in the country, the presence of great public health hazards of *Salmonella* [12]. A number of studies conducted by different individuals on various slaughtered beef animals and foods of beef origin are showed the prevalence of *Salmonella* in the country as indicated in the Table 1 below.

Incubation Period

The incubation period in animals is highly variable. In many cases, infections become symptomatic only when the animal is stressed. In horses, severe infections can develop acutely, with diarrhea appearing after 6 to 24 hours.

Clinical Findings

Illness usually appears when animals are stressed by factors such as transportation, mixing or crowding, food deprivation, weaning, parturition, exposure to cold, a concurrent viral or parasitic disease, sudden change of feed, or overfeeding following a fast. The clinical signs vary with the infecting dose, health of the host, *Salmonella* serovar and strain, and other factors. Some serovars tend to produce a particular syndrome. Although salmonellosis can be seen in all domesticated animals, pregnant, lactating or young mammals and birds are the most susceptible. The disease is most satisfactorily described as three syndromes, classified arbitrarily according to severity as septicemia, acute enteritis, and chronic enteritis.

Septicemia

This is the characteristic form of the disease in new born foals and calves, and in young pigs up to 4 months old. Commonly, there is profound depression, dullness, prostration, high fever (40.5-42°C, 105-107°F) and death within 24-48 hours.

Acute enteritis

This is the common form in adult animals of all species. There is a high fever (40-41°C) with severe, fluid diarrhea, sometimes, dysentery, and occasionally tenesmus. The fever often subsides precipitously with the onset of diarrhea. The feces have a putrid smell and contain mucus, sometimes blood, fibrinous casts, which may appear as complete tubular casts of intestine, and intestinal mucosa in sheets or casts. There is complete anorexia but in some cases increased thirst. The heart rate is rapid, the respirations are rapid and shallow and the

Table 1: Prevalence of salmonella in different parts of Ethiopia from 2003-2017.

Area	Species	Sample type	Prevalence	Year	Authors
Addis Ababa and Modjo	Sheep and goats	Faeces, mesenteric lymph nodes, liver, spleen, and abdominal and diaphragmatic muscle	1.80%	2003/2004	[42]
Modjo	Sheep and goats	Skin swabs, mesenteric lymph nodes, hand swabs, caecal contents, knife swabs, carcass and water	8.90%	2007/2008	[43]
Addis Ababa	Cattle	Faecal and milk	10.76%	2010	[44]
Addis Ababa Abattoir enterprise	Sheep and goats	Liver, kidney, spleen, muscle, carcass, mesenteric lymph node and feces	1.04%	2010-2011	[45]
Gondar	Cattle	Raw meat and swab	17.30%	2013	[46]
Holeta	Cattle	Rectal feces, udder milk, pooled milkers, hand swab, tank milk, tank swabs, and bucket swabs	5.60%	2014	[47]
Asella	Cattle	Carcass swab, Hanging material swab, Knife swab, Hand swab, lymph node, Faeces, milk	6.50%	2014	[48]
Gondar	Animal-origin food items	Raw meat, minced meat, burger, raw eggs, and raw milk.	5.50%	2014-2015	[49]
Eastern Hararghe	Sheep	Faeces	6.19%	2014/2015	[50]
Addis Ababa	Cattle	Fecal and carcass swab	3.70%	2014/2015	[51]
Dessie	Cattle	Meat, eviscerating knives and	4.95%	2014/2015	[52]
Bahir Dar	Cattle	Meat	70%	2015	[53]
Modjo and Bishoftu	Sheep and goats	Cecum, liver, mesenteric lymph nodes, abdominal muscle	17.21%	2015/2016	[54]
Eastern Haraghe	Cattle, sheep and goats	Faeces	5.07%	2015/2016	[55]
Holeta	Dogs	Rectal Swab	17.10%	2015/2016	[56]
Ambo	Cattle	Mesenteric lymph nodes and feces	8%	2015/2016	[57]
Wolaita Sodo	Cattle	Abdomen, thorax, crutch, and breast	12.50%	2015/2016	[58]
Addis Ababa	Cattle	Feces, carcass swabs, milk	7.50%	2017	[59]

mucosae are congested. Newborn animals that survive the septicemic state usually develop severe enteritis, with diarrhea becoming evident at 12-24 hours after the illness commences. If they survive this stage of the illness, residual polyarthritis or pneumonia may complicate the recovery phase [35].

Chronic enteritis

This is a common form in pigs following a severe outbreak, and occurs occasionally in cattle and adult horses. In calves there is intermittent or persistent diarrhea, with the occasional passage of spots of blood, mucus and firm fibrinous casts, intermittent moderate fever (39°C), and loss of weight leading to emaciation. Although chronic enteritis may occur initially it usually succeeds an acute episode [35].

Diagnosis

The clinical sign and finding at postmortem examination are not unique to salmonellosis although a tentative diagnosis may be made. Diagnosis of salmonellosis depends on clinical signs and isolation of the pathogen from feces, blood, or tissues of affected animals. When infection of the reproductive organs or abortion occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs and, in the case of poultry, embryonated eggs [11].

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, trypticsoy 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 [36].

Detection of antibodies by enzyme immunoassay (EIA)

The detection of antibodies to *Salmonella* by EIA offers a sensitive and cost effective method for mass screening of animal herds for indications of a past/present *Salmonella* infection. The advantage of this method is that it can be automated and no incubation is required to increase the numbers of bacterial cells. The well-established technique for assaying antigens is EIA. 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. Serological test, such as ELISA, serum agglutination and complement fixation can be used for the retrospective diagnosis of salmonellosis or the detection of carriers [37].

Molecular assays

Nucleic acid amplification methods have the potential to amplify small numbers of organisms and non culturable bacteria, as well as

dead organisms. Real-time quantitative polymerase chain reaction using PCR (Q-PCR), reverse transcriptase PCR (RT-PCR), and nucleic acid sequence-based amplification (NASBA) are used for detection of *Salmonella* from various food matrices [38].

Treatment, Prevention and Control Methods of Salmonellosis

Treatment consists of controlling the infection with effective antibiotics, and maintaining fluid balance with electrolytes. *Salmonella* in calves can be prevented or treated with anti-*salmonella* antibodies, and with fluid replacement therapy. Wise use of antimicrobials must be practiced to combat the ever increasing situation of antimicrobial resistance. Although many cases with salmonellosis recover without antimicrobial therapy, those with severe infections may require treatment; multidrug-resistant organisms limit effective medication choices [35].

Treatment always consists of providing energy and fluids; therefore electrolyte therapy is the most important treatment, whilst maintaining milk intakes as well. Specific treatment for *Salmonella* includes giving rotagen combo containing *salmonella* antibodies in the milk and the use of antibiotics if prescribed by your veterinarian. Severely affected calves may benefit from anti-inflammatory and some may require intravenous fluid therapy to survive. *Salmonella* is prone to developing antibiotic resistance so it is important that when calves are treated with antibiotics that the appropriate antibiotic, dose and length of treatment is given, otherwise resistance may occur [39].

No commercial vaccines are available. Biosecurity, external and internal, is a major focus in the control of *salmonella* in cattle herds. Good husbandry is important for good animal immune status. Animal movements are common within the cattle production sector with sales of calves, movement of heifers to and from rearing units, and animal trade between dairy herds [40].

Zoonotic Importance

Salmonellosis is a common human intestinal disorder primarily associated with *Salmonella*-contaminated meats and poultry [11]. Infections with *Salmonella* in food-producing animals present a serious public health concern, because food products of animal origin are considered to be a significant source of human infection. Most common sources of infection are eggs and related products, and meat from poultry and other food animal species. Milk and dairy products have also been associated with outbreaks of salmonellosis in people. In addition, contamination of fruit and vegetables by infected water may also be a source of infection [41].

Conclusion

Salmonellosis is a highly contagious bacterial disease of animals and human beings with significant economic impact. Human salmonellosis around the globe and these outbreaks have been linked with consumption of *Salmonella*-contaminated foods of animal origins such as poultry and related derived products, pork, cattle, sheep, goats, fish etc. *Salmonella* like many other enteropathogenic bacteria has evolved in utilizing a variety of virulence markers and other cellular machinery to colonize the host by attaching, invading and bypassing the host's gastrointestinal defense mechanisms. Animals are a primary reservoir for nontyphoidal *salmonellae*

associated with human infections, and contact with animal feces either directly through animal handling or manure or indirectly through fecal contamination of foods are principal vehicles of human infection. Animal health experts can be an important link to reducing the incidence of nontyphoidal salmonellosis in humans by assisting in the development and implementation of control strategies to reduce carriage of *salmonellae* by food-producing and companion animals.

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