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Bovine Salmonellosis and its Zoonotic Importance

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Abstract: Salmonella are widely considered as one of the most ubiquitous pathogens both in humans and animal; make up a large genus of Gram-negative bacilli, non capsulated, short rods and non spore forming within the family *Enterobacteriaceae*. The serovars most commonly cause bovine salmonellosis are *salmonella typhimurium*, *salmonella dublin* and *salmonella newport*. The epidemiology of salmonellosis is complex which often makes control of the disease difficult. The geographical distributions of the serotypes differ; *salmonella typhimurium* has universal distribution; while *salmonella dublin* has more patchy habitats. The source of environmental contamination is invariably feces and components of animal feeds. The feco oral route is the most important mode of transmission of Salmonella in animals and humans. Salmonellosis is of a common occurrence of domestic animals and the consequence of infection ranges from subclinical carrier status to acute fatal septicemia. There are numerous methods for isolation of *Salmonella* in use world-wide; such as culture, serological tests and molecular techniques. Salmonella serotypes are associated with three distinct human disease syndromes; bacteremia, typhoid fever and enterocolitis. The use of antimicrobials for the treatment of clinical salmonellosis is controversial. Prevention and control of salmonellosis is a major problem because of carrier animals and contaminated feedstuffs. Because of the risk of human infection, the meat production industry can be particularly negatively affected when a *Salmonella* infection occurs.

Key words: Bovine · Salmonellosis · Zoonoses

INTRODUCTION

Salmonellosis is a collective description of a group of diseases caused by bacteria of the genus salmonellae which is a member of the family *Enterobacteriacea* and is composed of two species (*Salmonella bongori* and *Salmonella enterica*) [1]. Salmonellosis is one of a common occurrence of domestic animals and the consequence of infection ranges from subclinical carrier status to acute fatal septicemia. The disease is most satisfactorily described as three syndromes classified arbitrarily according to the severity as septicemia, acute enteritis and chronic enteritis [2, 3].

Salmonella species are Gram-negative bacilli, straight rods, usually motile with peritrichous flagella (*Salmonella pullorum* and *Salmonella gallinarum* are non-motile exceptions), facultatively anaerobic that can reside in the intestinal tract of a wide variety of mammals, birds, reptiles and even insects [1, 4]. They are ubiquitous geographically and zoologically [5]. The epidemiological patterns of the prevalence of infection and incidence of disease differs greatly between geographic areas depending on climate, population density, land use, farming practice, food harvesting and processing technologies [3]. Persistence of infection in animals and in the environment is an important epidemiological feature of bovine salmonellosis. The source of environmental contamination is invariably feces [6]. The majority of the animals become subclinical excretors. However, *Salmonella* can survive for 9 months or more in the environmental sites such as moist soil, water, fecal particles, feeds especially in blood, bone and fish meat [7]. Feco-oral route is the most important mode of transmission in animal and humans [5].

Diagnosis is based on clinical suspicion. Hematologic and biochemical findings are non specific, being typical of infection diarrhea or septicemia [4]. A definitive etiological diagnosis of salmonellosis depends on culture of the

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organism from feces, blood, milk and other body fluids or tissues. Salmonellosis is a significant cause of economic loss of farm animals; because of the clinical disease cost which include death, diagnosis and treatment of clinical disease, diagnostic laboratory costs, cost of cleaning and disinfection and the cost of control and prevention [3]. The concern with the use of antibiotic is that they have the potential of predisposing to colonization with *salmonellae,* increasing levels and duration of excretion by carriers [1]. Control of salmonellosis is based on reducing the risk of exposure to infection by implementing a closed herd policy, purchasing animals from reliable source and preventing contamination of food staffs and water [8]. Therefore, the objectives of this seminar paper were to provide imperative points on the epidemiology and pathogenesis of bovine salmonellosis, to highlight preventive and control measures and its zoonotic importance.

Bovine Salmonellosis

The genus Salmonella: Salmonella was named after Salmon Elmer Daniel, an American veterinary bacteriologist, who described *Salmonella enterica* (Formerly *Salmonella Cholerasuis*), however, it was his colleague and subordinate Smith Theobald who first described the bacteria, from pigs, in an investigation for the cause of *hog cholera* [9]. The genus *Salmonella* belongs to the family *Enterobacteriaceae* and is divided into serovars based on the detection of three major antigenic determinants; the somatic (O), surface VI antigen (Capsular) and flagellar (H) antigen [10]. Currently there are 2463 serotypes of Salmonella and contains two species: S*almonella bongori* and *Salmonella enterica*. There are six subspecies with in *Salmonella enterica*, *enterica* (Sometimes designated as subspecies I), *salamae* (Subspp. II), *arizonae* (Subspp. IIIa), diarizonae (Subspp. IIIb), *indica* (Subspp. IV) and *houtenae* (*Subspp.VI*) (Those belonging to subspecies V were placed into *salmonella bongori* [5]. The serovars (Serotypes) that most commonly cause bovine salmonellosis are *Salmonella typhimurium, Salmonella dublin* and S*almonella Newport* [3]. Depending on host, the serovars are classified as either host adapted or non host adapted.

Characteristics of Salmonellae: *Salmonella* make up a large genus of gram-negative bacilli within the family *Enterobacteriaceae* and motile (With a few exception), facultatively anaerobic bacteria non capsulated, short rods (2 to4 by 0.5to0.1 micrometers) that have peritrichous flagella and also frequently carry fimbriae (*Salmonella gallinarum* and *Salmonella pullorum* do not have flagella) [12].

Table 1: Host adapted and non host adapted serovars of *salmonella*

Host adapted	Non host adapted
Salmonella Dublin	*Salmonella Newport*
Salmonella paratyphoid (A, C)	*Salmonella typhimurium*
Salmonella typhi	*Salmonella anatum*

Source: Timoney *et al*. [11].

Salmonella can grow on a defined media without special growth factors; colonies are usually 3 to 4 mm in diameter. But some serotypes produce small colonies (1mm) [11]. *Salmonella* can grow between 8°C and 45°C and at a pH of 4 to 8. The optimum growth temperature is 37°C. They do not survive at a temperature of higher than 70°C. The bacteria can resist dehydration for every long time, both in feces and in foods for human and animal consumption. In addition, they can survive for several months in brine with 20% salinity, particularly in products with high protein or fat content, such as salted sausages; they also resist smoking [12]. It has been indicated that they can survive for a long time in soil and water; in damp shaded soil for up to nine months [9].

Salmonellae usually produce gas from glucose and can use citrate as carbon source. They reduce nitrates to nitrite. Decarboxylase reactions are usually positive. Hydrogen sulfide (H_2S) is produced by most serotypes. Lactose is not fermented (Sometimes of *Salmonella arizonae* ferment lactose slowly, others ferment it rapidly) [11].

Epidemiology

Distribution: 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 differs greatly between geographical area depending on climate, population density, land use, farming practice, harvesting and processing technologies and consumer habits [3]. *Salmonella* species are ubiquitous geographically and zoologically [5, 13]. The disease has assumed major importance because of outbreaks in dairy cattle and the occurrence of infections in humans. Stressors that precipitate clinical disease include deprivation of feed and water, minimal levels of nutrition, long transport times, calving and antibiotic prophylaxis and mixing and crowding of feedlots [3, 13]. The geographical distributions of the serotypes differ. For example, *Salmonella typhimurium* has universal distribution; while *Salmonella dublin* has more patchy habitats [3].

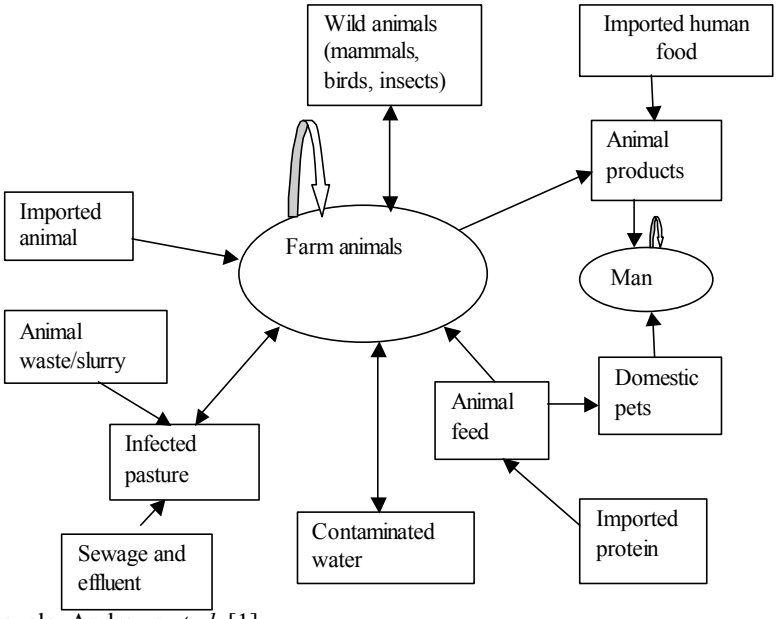

Fig. 1: The Salmonella cycle. Andrews *et al*. [1].

Host range and susceptibility: *Salmonella* have a wide variety of domestic and wild animal hosts. *Salmonella* serovars differ in the range of hosts they can infect and in the nature of disease that may result; this difference is referred as serovar host specificity [3]. Salmonellosis in adult cattle occurs sporadically, but in calves it usually acquires epizootic proportions. The disease generally occurs when stress factors are involved [12]. Some S*almonella* serovars, for example S*almonella anatum*, S*almonella newport*, S*almonella typhimurium* and *Salmonella enteritidis* can infect a wide range of hosts and are termed ubiquitous. Other serovars are very restricted in their host range causing sever systemic disease in only one host. For example, *Salmonella typhi* is restricted to infection in humans, *Salmonella abortus ovis* in sheep, *Salmonella dublin* in cattle, *Salmonella abortus equi* in horse and *Salmonella cholerae suis* in pigs [3, 14].

The disease usually affects young (4-6 weeks of age) as well as adult animals. Feedlots are commonly affected [5]. Host susceptibility may be related to immunological status, genetic makeup or age. Young and debilitated or aged animals are particularly susceptible and may develop the systemic form of the disease [6].

Source of Infection: The organism may persist in the soil, water, animal feces, raw meat, offal and vegetable materials. The source of environmental contamination is invariably feces and components of animal feeds (Bone, fish meal), particularly those containing milk, meat, or egg derived constituents and the feces of infected individuals [5]. In developing countries the source of infection is mainly the contaminated environment and water sources where animals crowed together. Contaminated equipment and utensils in the abattoirs are also source of infection [12]. Septicemic animals shed the agent in oral and nasal secretions and urine as well as feces. These animals do not necessarily have clinical signs associated with enteric salmonellosis at the time**.** Such animals are very dangerous because they contaminate water bowls, nipples, oral treatment equipment and human hands [6].

Mode of Transmission: The feco oral route is the most important mode of transmission of *Salmonella* in animals and humans [5, 6]. It can also occur through the upper respiratory tract and conjunctiva [1].

Carrier State: Because salmonellae are facultative intracellular organisms that survive in the phagolysosome of macrophages, they can invade the bactericidal effect of antibody and complement. Thus, persistence of infection in animals and in the environment is an important epidemiological feature of salmonellosis [3]. When an animal is infected with *Salmonella dublin,* it may become a clinical case or an active carrier shedding organisms constantly or intermittently in the feces. It may also become a latent carrier with infection persisting in lymph nodes or tonsils. But no *salmonella* in the feces or even a passive carrier which is constantly acquiring infection

from pasture or calf pen floor [1]. For *Salmonella typhimurium* the donor can be any domestic animal species, including humans or any wild animals or birds. Although all infected adults become carriers. It is rarely for any length of time and calves rarely become carriers [3].

Pathogenesis: Pathogenesis of salmonellosis is a complex and multifactorial phenomenon. Following ingestion, a proportion of the organism resists the low pH of the stomach, reach the distal ileum and the cecum, invade the mucosa and replicate in the submucosa and payers patches. In young and adult animals whose resistance has been lowered, spread beyond the mesenteric lymph node occurs and the infection is established in the reticulo endothelial cells of the liver; from there it invades the blood stream. A febrile reaction follows in 24-48 hours and the acute phase of the disease is present 3-9 days. Later the early septicemia may be rapidly fatal. If the septicemic invasion is sufficient to cause only bacteremia, acute enteritis may develop and abortion is a common final sequel in sheep and cattle [3].

After passage through the stomach, surviving Salmonella must be able to resist several host innate defense, including lyzosyme and lactoferin and to prevent their removal from the intestines in the normal movement of food by peristalsis [1]. Entry of *Salmonella* usually occurs without mucosal damage in systemic infection, but enteric infection is characterized by local damage without septicemia. *Salmonella* interaction with M cell in payer's patches is facilitated by fimbrial adhesion. This is followed by ruffling of the target cell membrane, which results in internalization of bacteria in membrane bounded vacuoles. Invasion is followed by inflammation, with a notable profusion of neutrophils as a result of production of IL-8 at the basolateral surface and of an epithelial chemoatractant at the apical border. Neutrophils migrate into lamina propria and then to the lumen. Disruption of tight junctions permits access to receptors on the basolateral surface, increasing production of pro inflammatory cytokines [15].

Cell death and sloughing allow bacterial invasion of the sub mucosal tissues. M cells transcytosis brings the organisms into contact with host phagocytes. Some macrophages are killed rapidly, in a process that shares features of apoptosis and necrosis. Intraphagosomal survival is required for establishment of *Salmonella* species in the intestine and for systemic spread. Survival and replication in phagocytes and subsequently in lymph nodes can lead to extra intestinal dissemination. Toxic effect of lipopolysaccharide (LPS) results in over stimulation of the host cytokine response and the ultimate effects include inflammation, shock, fever and death [15].

Clinical Signs: Salmonellosis is one of a common occurrence of domestic animals and the consequence of infection ranges from subclinical carrier status to acute fatal septicemia [6, 12]. The disease is most satisfactorily described as three syndromes, classified arbitrarily according to the severity as septicemia, acute enteritis and chronic enteritis [3, 6, 15].

Septicemic Form: Septicemic form occurs in a new born foal, calves and in young pigs up to four months old. Commonly there is profound depression, dullness, prostration, high fever (40.5°C- 42°C) and death within 24-48 hours [3]. New born animals that survive the septicemic state usually develop sever enteritis, with diarrhea becoming evident at 12-24 hours after the illness commences [1].

Enteric Form: Acute enteritis is the common form in adult animals of all species. There is a high fever (40-41°C) with sever 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 a complete tubular casts of intestine and intestinal mucosa in sheets or casts. There is a complete anorexia but in some cases increased thirst, rapid heart rate, rapid and shallow respiration, congested mucosa, severe dehydration and toxemia, weight loss, become recumbent and dies within 2-5 days. Abortion in pregnant animal, abdominal pain, kicking at the abdomen, rolling, crouching, grooming and looking at the flanks may occur in adult cattle [1, 3].

Chronic Form: Chronic enteritis with inappetence, reduced weight gain and unthriftiness may follow from an attack of acute enteritis or be the only manifestation of the disease [16]. Abortion is a common sequel in pregnant cows that survive an attack of acute enteritis. However, infection with *Salmonella dublin* is also a significant cause of abortion in cattle without their having been only clinical sign other than retained placenta. A sequel to some cases of apparent enteric salmonellosis is the development of terminal dry gangrene due to end arteritis of the extremities, including ear tips, tail tips and the limbs from the fetlock down [1, 3, 16].

Table 2: Characteristics of *Salmonella* colonies on different media

Medium	Colonial characteristics
MacConKey agar	Colorless to grayish colonies
Brilliant Green agar	Red colonies
Xylose- lysine agar	Pink-red colonies with black centers
Hekton enteric agar	Green colonies with black centers

Source: Timoney *et al*. [11]

Necropsy Findings

Septicemic Form: Gross lesions of *Salmonella* infections are acute and usually subtle. Serosal and subcutaneous petechiae are wide spread. The spleen is usually enlarged, the lungs are edematous and felt to collapsed and have random foci of hemorrhage or congestion. Less common lesions include jaundice, cystitis, meningitis, osteomyelitis and arteritis [16].

Enteric Form: The most consistence damage is found in the large and small intestine. The character of the inflammation here varies from mucoenteritis with sub mucosal petechiation to diffuse hemorrhagic enteritis [16]. The abomasum usually contains brown, fetid liquid and bowel contents are watery and may contain fibrin or blood or both. The mesenteric lymph nodes are frequently enlarged and dark [3].

Chronic Form: The chronic form is usually 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 surface [16].

Diagnosis

Diagnostic Techniques: There are numerous methods for isolation of *Salmonella* in use world-wide. Some of the more common methods are culture (Pre-enrichment media, enrichment media, selective plating media), serological tests (Serum agglutination test, rapid slide agglutination test, enzyme-linked immunosorbent assays), molecular techniques (PCR).The culture techniques and media that may work best in a particular diagnostic situation depend on a variety of factors, including the *Salmonella* serovar, source and type of specimens, animal species of origin, experience of the microbiologist and availability of selective enrichment and selective plating media [3].

Differential Diagnosis: The clinical diagnosis of salmonellosis is difficult because of a number of other diseases that resemble each form of the disease. The septicemic form of salmonellosis in calves resembles coliform septicemia and differentiation is possible only by bacteriological examination of blood, feces and tissue. Salmonellosis occurs most commonly during the second and third weeks of life in contrast to coliform septicemia which occurs most commonly in the first few days of life. Acute enteritis resembles coccidiosis, acute intestinal obstruction, winter dysentery, mucosal disease, bracken fern poisoning and the chronic form of bovine salmonellosis resembles John's disease or chronic molybdenum poisoning, but dysentery and epithelial casts do not occur in these disease [3, 7].

Treatment: The use of antimicrobials for the treatment of clinical salmonellosis is controversial and different approaches to the problem exist among veterinarians. Ceftiofur at 5mg/kg BW IM/24 hours is effective for neonatal calves and sulfonamides for adult [3]. Development of drug resistant by *Salmonella* raises further public health concerns. In fact, the *Salmonella typhimurium DT104*, which exhibits a resistant pattern to multiple antibiotics including ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline [17].

Control and Prevention: Prevention and control of salmonellosis is a major problem because of carrier animals and contaminated feedstuffs. Currently a major effort is being attempted to control *Salmonella* infection in production facilities around the world. In several countries, the application of the hazard analysis critical control point (HACCP) methodology is being used at meat processing facilities to control pathogen introduction into the food supply [18].

The principles of control include preventing the introduction of carrier animals by maintaining a closed herd policy or by purchasing from a herd of a known health status [17]. Avoid the use of hospital pens for processing of newly arrived animals, pay careful attention to the cleanliness of feed and water sources; restrict the movement of cattle and personnel handling cattle so as to prevent the spread of the disease; isolate sick animals as much as possible because they are shedding large numbers of bacteria that quickly contaminate the environment [6, 13], dispose of carcasses by burning or burying in a timely manner to prevent further spread of disease by wild and domestic animals or water runoff; regular cleaning of all facilities in the processing area and the hospital pens; access to feed storage areas by possible carrier animals, such as wild birds and mice, as well as dogs and cats, should be prevented; pets, such as dogs and cats, should have restricted contact with

livestock and animal facilities; prevention of *Salmonella* using vaccines has shown mixed results and cannot be universally recommended at present [3, 19].

Zoonotic Importance of Bovine Salmonellosis: Salmonellosis is perhaps the most wide spread zoonosis in the world [20]. The disease situation in human has become increasingly worse during the last decade. According to WHO, the situation has reached alarming proportion in several countries. This is largely as a result of the industrialization and large scale intensive production of livestock which has opened the door to the food chain of zoonoses like salmonellosis which cannot be controlled by the traditional post mortem inspection [18]. Animals are the reservoir of zoonotic salmonellae*;* especially any food of animal origin can be source of infection [12]. With the exception of S*almonella typhi* and S*almonella paratyphoid* serotypes particularly A and C, which are specific for man, all other infections caused by *Salmonella* may be considered as zoonoses [12, 21]. Persons at risk are those in contact with animals and animal products, particularly animal dealers, farmers, animal keepers and personal (In food industries and restaurants where animal products are processed) [22].

Salmonella live in the intestinal tract of humans and other animals, including birds and are transmitted to humans by eating foods contaminated with animal feces and are often of animal origin, such as beef, poultry, milk or egg. But any food, including vegetables, may become contaminated [13]. Salmonella serotypes are associated with three distinct human disease syndromes, bacteremia, typhoid fever and enterocolitis. Of these, bacteremia, a syndrome caused by the porcine-adapted S. enterica serotype *Cholerae suis* and the bovine-adapted S. enterica serotype *dublin*, is encountered least frequently in humans. Salmonella-induced enterocolitis is the single most common cause of death from food-borne illnesses associated with viruses, parasites, or bacteria in the United States [23]. Cutaneous salmonellosis has been reported in veterinarians attending in infected cattle at the time of parturition. The disease is characterized by pustular dermatitis from which *Salmonella virchow* and S*almonella dublin* were isolated. Veterinarians my develop skin lesions after obstetric deliveries, even after hygienic precautions and careful washing of the arms and hands [3].

Most often, salmonellosis is characterized by nausea and vomiting within 8-48 hours of ingestion. Shortly thereafter, the patient will experience abdominal pain, cramps or diarrhea. Patients may have moderate fevers (Up to 102°F) and occasional chills. More severe symptoms may occur in higher risk groups of people, such as the very old or young, those with impaired immune systems (AIDS, arthritis, transplant or cancer patients), those who already have a gastrointestinal disorder, or those receiving antibiotics. In higher risk groups of people, *Salmonella* may invade beyond the gastrointestinal tract and cause severe systemic illness; such as more extreme nausea, prolonged fever, chronic diarrhea, overall lethargic feeling and in some cases, death. Consequently, people with these conditions should take special precautions, especially when working around calves or other high-risk animals [3, 12].

How sick an infected person will depend on several things, including the serotypes of *Salmonella,* the number of organisms ingested and how well the person's body (Immune system, gut function and state of health overall) is equipped to handle the infection. Some individuals are considered to be at high risk just due to their age or health status [24]. Zoonotic salmonellosis normally has a benign course and usually heals without complications and the only treatment recommended is rehydration and electrolyte replacement [12, 24].

The most serious risk is that the transmitted bacteria will have acquired resistance to specific antibiotics because the animals from which they originate have been treated with the particular antibiotics repeatedly or a long period [3]. Consumers should not eat raw or undercooked ground beef. Wash hands, kitchen work surface and utensils with soap and water immediately after they have been in contact with raw meat [25].

CONCLUSIONS

Salmonellosis is a collective description of a group of disease caused by bacteria of the genus Salmonella. *Salmonella* have a wide variety of domestic and wild animal hosts and occur worldwide. The epidemiological patterns of disease differ greatly between geographic areas depending on climate, population density, land use, farming practice, food harvesting and processing technologies. It can survive for nine months or more in the environmental sites such as moist soil, water, fecal particles, feeds especially in blood, bone and fish meat. In developing countries the source of infection is mainly the contaminated environment and water sources where animals crowed together. The use of antimicrobials for the treatment of clinical salmonellosis is controversial and different approaches to the problem exist among

veterinarians. Prevention and control of salmonellosis is a major problem because of carrier animals and contaminated feedstuffs.

Therefore, based on the above conclusions the following recommendations are forwarded:

- Further detailed studies involving different sources and different types of food animals on the contamination levels, diversity of serovars and antimicrobial resistance of *Salmonella* should be done.
- Personal hygiene and food sanitation should be practiced to prevent infection from different sources.
- Awareness should be created among public about the risks associated with consumption of raw and under cook meat for prevention of human salmonellosis.

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