Sudan University of Science and Technology

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Prevalence and Risk factors of Salmonella Infection in cattle in Omdurman

انشار الإصابة وعوامل الخطر لمرض السالمونيلا في الأبقار في ام درمان

By

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Dedication

To my husband for support me.

To my parent.

To all my brother and sisters for making everything wonderful.

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Acknowledgment

Firstly, praise to almighty Allah for giving me strength to finish this work. I would like to thank my Supervisor prof. Mohamed Abdelsalam Abdalla for his kind guidance and close fruitful supervision at any time when ever needed. Thank are also due to Dr.Olaa helped in the design data analysis of the questionnaire survey. I also would like to thank the technical staff especially Najwa .

Abstract

Asurvey was carried out to estimate the prevelance of *Salmonella* spp and risk factors in Omdurman localities (Karari,Ombada and Abosyede)-Khartoum state.Atotal 0f 158 random samples of feces(100 samples and milk 58 samples) were collected from cattle.Isolation and identification of *Salmonella spp* were done.The total viable count(TVC)was also used.Sixty three out of 158 collected samples were positive to *Salmonella spp*.In fecal samples 40% were positive for isolated bacteria .The higest infection in milk samples were 44% in Karari and lowerst infection 35% in Abosyede.Statistically ,there was no significant difference($p \leq 0.05$).The TVC revealed the highest contamination in fecal samples (2.00±1.22 log 10 cfu/g) while lowest contamination in milk(2.09±0.40 log 10 cfu/g).Risk factor that showed significant association in *Salmonella* infection was source of animals (OR=0,p-value=0.04,CI95%=0.00-0.95) and using outside bulls(OR=33,P-value=0.04,CI95%=1.04-1042.24).The presence of relative high level of *Salmonella ssp* in fecal samples and milk in this locality indication of bad hygienic status in these dairy farms.

الخلاصه

اجريت هذه الدراسه لتحديد نسبه الاصابه لمرض السالمونيلا والعوامل المساعده في منطقه امدرمان(كررى-امبده-ابو سعد)ولايه الخرطوم في الفتره من سبتمبر الي اكتوبر 2013 وكان العدد الكلي للعينات 158 عينه اختيرت عشوائيا (100عينه براز و58 عينه لبن) من الابقار.تم عزل انواع السالمونيلا في المعمل . ثلاثه وستون عينه من 158 عينه كانت ايجابيه .اعلي نسبه اصابه في اللبن كانت في كرري(44%) واقل نسبه اصابه كانت في ابوسعد(35 اما نسبه الاصابه في البراز كانت (40%).احصائيا لايوجد فرق معنوي($p\leq0.05$).العدد الكلي للبكتريا كان اعلي تلوث في البراز(2.00±1.22log10cfu/g)بينما اللبن اقل تلوث(2.09±0.40log10cfu/g).وجد ان هناك فرق معنوي لمصدر الحيوانات(OR=0,Pvalue=0.04,CI95%=0.00-0.95) والاستخدام الحارجي للثيران.(OR=33, P-value=0.04 CI95%=1.04-1042.24) . وجود السالمونيلا بهذه النسبه العاليه في منطقه امدرمان يدل علي سؤالتحكم الصحي في مزارع الالبان.

Introduction

Salmonella is a gram-negative,non spore forming, rod shaped, facutative intracellular anaerobic bacterium in the family *Enterobacteriaceae* trivially known as entric bacteria (Todar,2005).They are cytophilic ,non capsulated bacilli and are motile with peritrichous flagella ranging in size from 2to 3 by 0.6 (Paul and colin ,1990).They metabolize glucose to acids , catalaze –positive, oxidaze –negative. However, They can also live under aerobic condition.*Salmonella* lives in the intestinal tract of warm and cold blooded animal i,e humans and animals(Todar,2005).

Salmonella Typhimurium is the most widely distributed bacteria that cause enteric fever or gastro entritis which is designated as food poisoning and it transmitted generally through contaminated food and water (Schlegel, 2002). Prevalence of *Salmonella* since 200 the number of cattle herds with Salmonella infection has varied between 4 and 13 per year. In 2008, 21 serotype were detected with *Salmonella* and most common serotype is *S. Dublin and S. Typhimurium* (SVA , 2011) . Syptoms of *Salmonella* infection in cattle vary and in many infected animals show no syptoms , this depend on serotype. Syptoms that can be seen are diarrhoea,abortion,fever,poor general condition,decreased appetite,pnemonia and death.(SVA,2011).

Several well recognized pathogens such as *Salmonella, Escherichia* coli 0 1 57: H7, *Campylobacter, and Yeninia enterocolitica* ،have emerged as public health problems and have increased in prevalence or become associated with new food vehicles (Altekruse *et al*,1998). Among the various pathogenic bacteria known to cause mass food poisoning incidents, the most notorious are members of the genus *Salmonella* (Krieg and Holt, 1984). They are highly adaptive and potentially pathogenic to humans and animals. They are the causative agents of

enteric fevers, gastroenteritis and septicemia which are of both socio-economic and public health importance. This therefore makes the genus of *Salmonella* one of the most important bacteria zoonoses in the world. The disease prevalence is increasing as a result of industrialization and intensive production of livestock and poultry through small scale farmers (Nielsen, *et al*, 2007).In addition, selective pressure imposed by the use of antimicrobials in both human and veterinary medicine promotes the spread of multiple antimicrobial resistance resulting in the growing problem of Salmonella infections that are difficult to treat (Carattoli, 2003).

Salmonellosis is a significant foodborne zoonosis and public health issue worldwide .In Sudan, the prevalence of *Salmonella* serovars in humans and animals is not well documented, as *Salmonella* are not routinely isolated and identified. Only a few studies of *Salmonella* isolates from animals in Sudan have been published (Soliman and Khan ,1959; Khan,1961; 1962, 1970, 1971; Khogali *et al*, 1973; Mamoun *et al*, 1992; El Tom *et al*,1999). AbdelRahman *et al*, (1995) reported the isolation of *Salmonella* serovars *Dublin* and I:13, 23:b, a monophasic variant from cattle.

Objectives

1-To estimate prevalence of *Salmonella* species in Omdurman locality.
2-To investigate risk factors.

Chapter One

Literature review

1.1 General Overview of Bovine Salmonellosis

1.2 Classification

The current nomenclature of the genus Salmonella adopted worldwide through different publications is the one used by (CDC, 2008) is based on the recommendations from the WHO collaborating centre and it adequately addresses the concern and requirements of clinical and public health microbiologists (Olsen and Skov 1994].

Scientifically Salmonella classification is described under:

Domain: Bacteria

Phylum: Protobacteria

Class: Gamma Protobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: Salmonella

Species: Salmonella enterica and Salmonella bongori (Gyles, 2004]. The species of Salmonella enterica being divided into six subspecies (I–VI); *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S.* enteric subsp. *arizonae* (IIIa), *S. enteric* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV) and *S. enterica* subsp. *indica* (Kingsley et al, 2000]. 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(Vargas,2007].

Salmonella species are a group of Gram-negative enterobacteria and known human pathogens in developing as well as industrialized countries. Despite significant advances in sanitation, provision of potable water, and highly controlled food chain surveillance, transmission of *Salmonella spp*. continue to affect communities, preferentially children, worldwide. This review summarizes updated concepts on typhoidal and non-typhoidal *Salmonella* infections, starting with a historical perspective that implicates typhoid *Salmonella* as a significant human pathogen since ancient times. We describe the epidemiology of this pathogen with emphasis on the most recent non-typhoidal *Salmonella* outbreaks in industrialized countries and continued outbreaks of typhoid *Salmonella* in underserved countries. An overview of clinical aspects of typhoid and non-typhoid infections in developing and industrialized countries, respectively, is provided, followed by a description on current treatment concepts and challenges treating multidrug-resistant Salmonella infection

1.2.1 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(Magnino *et al*, 2009). *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(Hanning *et al*, 2008]. They are also able to multiply in the environment with low level or no oxygen(Gantois *et al*,

2008]. 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 (Padungtod *etal*, 2008) .

Salmonella spp. are Gram-negative, facultative e anaerobe rods with more than 2500 known serovars that belong to the family Enterobacteriaceae. Salmonella spp. are of high importance in food safety being able to provoke severe intestinal infections in humans which can lead to death especially in elderly people (KLEER, 2004). As in most animals, *Salmonella* infections are common in camels in countries all over the world. Whereas some of the affected animals show clinical symptoms; others do not

KLEER (2004) proved the presence of a serological reaction to *Salmonella* (S.) Typhimurium and S. Enteritidis antigens each in 5.8 % of the examined camels. No cases of lactogenic transmission from camels to humans have yet been reported .The most frequent reason for the presence of *Salmonella* spp. in milk is through faecal contamination after heat treatment as *Salmonellae* are inactivated during pasteurisation (KLEER, 2004).

Salmonella strains are serologically classified using Kauffmann- White scheme, and at the present the genus contains more than 2500 serotypes(Uzzau *et al,* 2000; Lammerding,2006). 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 antigns(Dalton *et al*, 2004; Bowen *et al*, 2006). Recently the number of Salmonella serotypes that

causes disease in human now approaches 3000 new serotypes are identified continuously(De Buyser *etal*, 2001].

Since *Salmonella* is closely related to both human and animal health, more rapid and sensitive methods for the identification of this bacterium are required (Schrank et al., 2001).In the regions where enteric fever is common, clinical diagnosis of typhoid fever is inadequate, as the symptoms it causes are non-specific and overlap with those of other febrile illness. Serological tests, predominantly the Widal test, are available but have very low sensitivity and specificity, and no practical value in endemic areas despite their continued use (Levine et al., 1978). Isolation of the causative organism remains the most effective diagnostic method in suspected typhoid fever (Zhou and Pollard, 2010). Standard culture methods for detecting *Salmonella* spp. include non-selective pre-enrichment followed by selective enrichment and plating on selective and differential media (Whyte et al., 2002). These methods take approximately 4-7 days (Harvey and Price, 1979) so they are considered laborious and time consuming.In a clinical setting, suspected Enterobacteriaceae are often subjected to biochemical testing to investigate the ability of an isolate to grow upon certain substrates, produce various metabolic products or alter the pH. In the 1970s, efforts were being made to collect together multiple biochemical tests to allow rapid and relatively high throughput identification of clinical bacterial isolates (Lindberg et al., 1974). Numerous test kits of varying accuracy became available for the identification of the Enterobacteriaceae. The API 20E system (BioMérieux, France) was found to be the most reliable, having a 99% correlation with standard biochemical tests and a 94% identification rate (Nord *et al*., 1974). Over 60 bacterial species can

currently be identified with the API20E, with identification extending to the serovar level for *Typhi* and *Paratyphi* A among others. However, results of such tests are open to interpretation and require experience and a high level of technical skill to generate reproducible results (Jamshidi *et al*., 2007). Therefore, several alternative faster methods for the detection of *Salmonella* have been suggested.

1.2.2 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 serovars differs so widely that *Salmonella* infection or *Salmonella* contamination are inevitably complex (Padungtod *et al*, 2008] .

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1.2.3 *Salmonella* and Transmission Dynamics

Salmonella serotypes can be divided into host restricted, host specific, and generalists serotypes, with important implications for epidemiology and public health (Uzzau, et al 2000]. Host specific serotypes, for instance serotypes *Paratyphi* A, *Gallinarum* biovars *Gallinarum* and *Pullorum*, or *Typhi* only cause disease in one host species(Uzzau, *et a*l 2000; Baumler, *et al* 1998]. In contrast, host restricted serotypes are predominantly associated with one host species, but can cause disease in other species as well(Uzzau, *et al* 2000). *Salmonella Dublin*, for example, is adapted to cattle but infections in small ruminants, pigs and humans have also been documented, and serotype *Choleraesuis* is adapted to

swine but has also been isolated from a range of other host species(Smith and Jones; 1967 Olsen and Skov, 1994]. Generalist serotypes such as *Salmonella Typhimurium* commonly cause disease in a broad range of hosts, even though a narrow host range has been described for certain subtypes, for instance *Typhimurium* subtypes DT2 and DT99, which appear to be adapted to pigeons(Gyles, 2004]. Serotypes with broad and narrow host range seem to differ in clinical manifestation even though other factors such as host species, age, and concomitant disease affect the clinical manifestation as well (Gyles, 2004) for a comprehensive review. Infections with generalist serotypes are often characterized by high morbidity but low mortality, and gastro-intestinal symptoms are the predominant clinical manifestation(Uzzau, et al 2000;, Gyles, 2004]. On the contrary, infections with host adapted or restricted serotypes, such as *Choleraesuis*, *Abortusequi*, *Gallinarum* biovar *Gallinarum*, or *Gallinarum* biovar *Pullorum*, are typically characterized by low morbidity and high mortality, and systemic disease is common(Uzzau, et al 2000;, Gyles, 2004].

Salmonella serotypes clearly seem to differ in their pathogenic potential for humans and serotype distributions often vary vastly between human and animal populations as well as among different animal populations in the same geographic area. For instance, approximately 40% of all known *Salmonella* serotypes are predominantly associated with reptiles or amphibians, yet less than 1% of human salmonellosis cases are caused by these reptile-associated serotypes. The molecular determinants of serotype-specific host range differences have so far largely remained elusive. However, serotype-specific differences in virulence have been characterized in some cases. For instance, in competition experiments with *Salmonella typhimurium*, reptile-associated *Salmonella arizonae* and

diarizonae showed a significantly reduced ability to colonize and persist in the intestine of BALB/c mice, clearly suggesting virulence differences(Kingsley et al ;2000).

Majowicz *et al* (2010) recently estimated that approx. 80.3 of 93.8 million human Salmonella-related gastroenteritis cases that are estimated to occur globally each year are foodborne, thus representing approx. 86% of human salmonellosis cases. Another study based on formal elicitation of expert opinion estimated that approx. 55% (range 32-88%) of human Salmonella cases are foodborne, 14% (range 3-26%) are travel-related, 13% (range 0-29%) are acquired through environmental sources, 9% (range 0-19%) occur due to direct human-to-human transmission and 9% (range 0-19%) are attributable to direct animal contact(EFSA, 2008; Vargas, 2007]. Yet another study, based on surveillance data, estimated that 95% of non-typhoidal human *Salmonella* cases in the USA are foodborne, emphasizing the complexity and controversy of the subject matter (Mead, et al 1999]. The comparison of *Salmonella* outbreak and surveillance data across geographic regions or from different time periods represents a considerable challenge. The advent of novel molecular subtyping methods has noticeably improved discriminatory power, with important impacts on sensitivity and specificity (Van Belkum, *et al* 2007). The impacts of differences in public health infrastructure, disease surveillance sampling plans, public health legislation etc. are difficult to measure, but differences in apparent prevalence between states or countries are clearly noticeable. Case and outbreak definitions are also variable. For clarity, we will henceforth define "case" to refer to any instance where strong epidemiological or molecular evidence suggests an animal source of human infection, while trying to point out instances where such conclusions are solely based on serotype data or

circumstantial epidemiological links. Furthermore, we will define an outbreak as an event where two or more human cases are presumably linked to the same source. We acknowledge that the epidemiological definition of an outbreak differs from this definition, but due to the large amount of underreporting and the problem of attributing cases with broad host-range serotypes to animal sources we chose this definition.

As some serotypes are strongly associated with specific animal species and some case definitions utilize serotype data to define for instance reptile-acquired cases (see for instance (Wells*, et al*2004; Ackman*, et al* 1995).

Infection routes and epidemiology on the farm The most important infection route in animals is the faecal-oral (Gillespie and Timoney ,1981).Respiratory infection is also possible; *Salmonella* may be spread by aerosols with the use of pressure hoses when cleaning stalls (Wray 2000). Contaminated milk may also cause infections. Occasionally cattle may excrete Salmonella in their milk, but more frequently the milk is contaminated by infected faeces during the milking process (Wray 2000). Newborn calves are exposed to *Salmonella* infection as the carrier cows shed th organism at parturition (Gillespie and Timoney, 1981). To cause disease in healthy cattle, oral doses ranging from 106 to 1011 cells of *S. Dublin* and 104 to 1011 cells of S. *Typhimurium* are needed (Wray 2000). However, infection has been seen even after the ingestion of very small doses (less than 10 cells). These infections were associated with ingestion of chocolate in humans and a vegetable fat supplement in cattle (Kapperud *et al*, 1990; Jones *et al*, 1982). The transmission of infection from one farm to another is mainly achieved by the purchase of infected cattle (Wray 2000). Non-symptomatic ruminants shed the bacteria

intermittently and therefore infection is difficult to detect (Edrington *et al*, 2004). Some asymptomatic carriers continue to eliminate Salmonellae in their faeces for weeks, months or years after recovery from a clinical case: and by doing so these carriers contribute to the disseminationof salmonellosis (Brenner 1984). Latent carriers are important in the epidemiology and persistence of *S. Dublin* on farms, because this bacterium can survive in the environment for over a year (Gillespie and Timoney, 1981; Wray 2000). Furthermore, *S. Typhimurium* has been shown to persist in calf rearing units for up to two years, despite cleaning and disinfection routinely carried out between batches (Wray 2000). Free-living animals, birds, and rodents may also introduce the infection onto the farm (Gillespie and Timoney 1981; Wray 2000).Cats, dogs, mice, rats, foxes and badgers have been shown to be infected with *S. Typhimurium*. Rats and mice that acquire *S. Dublin* infection do not play a major role in the spread of infection, but they might prolong the persistence of *Salmonella* on farms Wray *et al*, 2000) .

1.3Cattle and *Salmonella*

The clinical and economic importance of *Salmonella* infections among cattle abortions attributable to *Salmonella* infection are possible but rare in cattle, thus economic losses in cattle operations are primarily due to increased mortality, performance losses, and direct and indirect costs associated with treatment and infection control. Mortality rates attributable to Salmonella infection are particularly high in young animals, which also generally require the greatest amount of treatment. Significant weight losses in calves due to *Salmonella* infection have been reported in numerous studies, even though surviving calves seem to

regain the weight after recovery. In addition, Salmonella infection often leads to increased feed costs due to diminished feed conversion (Gile et *al* , 1989]

1.3.1Clinical Signs of salmonellosis

Clinically, the infection in cattle is typically manifested as watery or bloody diarrhea, and often associated with fever, depression, anorexia, dehydration and endotoxemia. Less common clinical manifestations include abortion and respiratory disease, and mortality rates can be high. Particularly in adult animals *Salmonella* frequently causes subclinical disease, and is known to persist on infected farms for months or years(Giles *et al*, 1989; Huston *et al*, 2002; Vanselow *et al*, 2007; Wray *et al*, 2000). Individual animals shed *Salmonella* intermittently, over variable periods of time, and infections with host adapted serotypes such as *Salmonella dublin* may potentially result more frequently in the development of asymptomatic shedders than infections with broad host-range serotypes(Brackelsberg *et al*, 1997]. One recent study estimated the median duration of shedding in dairy cattle to equal 50 days, with a maximum duration of 391 days, and the results appear comparable to previous reports(Cummings *et al*, 2009; Cobbold *et al*, 2006]. However, the duration of *Salmonella* persistence in herds exceeds maximum shedding durations observed for individual animals, and is believed to be largely attributable to endemic Salmonella infections within the herd(Cobbold *et al*, 2006). Several studies report isolating *Salmonella* at high rates from farm environments, a likely important Salmonella reservoir (Vanselow *et al*, 2007; Cobbold *et al* 2006; Clegg1 *et al*, 983 Fossler *et al*, 2005; Murray, 1991).

Salmonella within- and between-herd prevalence estimates vary considerably, with between-herd point prevalence estimates for cattle operations ranging from 2-42% and within-herd estimates for these operations ranging from 0-37%(Cobbold *et al*, 2006, Blau *et al*, 2005; Callaway *et al*, 2005; Ersboll *et al*, 2008; Huston *et al*, 2002; Smith et al, 1994; Lailler *et al*, 2005; Warnick *et al*, 2001). In addition, herds with clinically sick animals are generally high within-herd prevalence than herds where clinical salmonellosis is absent, and serotype distribution may differ between herds with and without clinical cases(Davies et al, 1998; . Letellieret al, 1999; Bahnson et al, 2006; Merialdi et al , 2008).Large herd size represents an important risk factor for salmonellosis, and the risk of *Salmonella* shedding seems to vary by production system, housing type, general hygiene level, management type and animal age, although the results reported in the literature have been somewhat contradictory(Nollet *et al*, 2004]. Calves, heifers, and periparturient cows generally appear to be at a particular risk of infection, and one study found heifers and periparturient cows to be the most likely cattle to become asymptomatic carriers(Cobbold *et al*, 2006; Nielsen *et al* 2004; Evans *et al* 1996).

The distribution of Salmonella serotypes among cattle varies greatly over time, and differs among geographic regions, age groups, clinical manifestation, and production systems. The United States National Veterinary Service Laboratory (NVSL), for instance, reported that serotypes *Typhimurium*, Newport, Orion, Montevideo, and Agona were the serotypes most frequently isolated from clinically sick cattle in 2005 and 2006, while serotypes Cerro, Kentucky, Anatum, Newport, Montevideo, and Orion were the serotypes most frequently isolated from clinically healthy cattle in the same time period.

1.3.2The global distribution and economic importance of mammalian livestock

The estimated number of mammals farmed for agricultural purposes around the world exceeds 4 billion animals (FAO, 2010]. In 2006, approx. 20% of the world's population, equaling approx. 1.3 of 6.55 billion people, were employed in the livestock sector, and livestock accounted for approx. 40% of global agricultural output(Steinfeld et al 2006]. An estimated 1 billion pigs and 2 billion small ruminants are farmed worldwide (Tomley *et al*, 2009]. The global cattle population is believed to equal approx. 1.3 billion animals, and 181 million buffaloes as well as approx. 24.7 million camels are farmed for commercial purposes around the world(FAO, 2010]. The relative and absolute abundance of livestock differs considerably by country and geographic area. For instance, the USA is the world's largest producer of beef and the third-largest producer of pork, while large numbers of cattle and pigs are also farmed in the EU(FAO, 2010]. Australia is a leading producer of wool and sheep meat, and India is an important producer of goat, buffalo and cow milk and a leading producer of goat and buffalo meat. Africa as well as parts of Western Asia are major producers of camel and goat products FAO, 2010)

1.3.3Treatment

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 using 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. (lindberge et al 1974)

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, trimethoprimsulfamethoxazole and newer fluoroquinoazoles 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 (Lazarus *et al*, 2007).

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 (Huston *et al*, 2001] .

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(Letellier *et al*, 1999). 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(Davison, 2005).

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1.4Prevention and Control

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 feacal contamination of the entire premise. When salmonellosis has been confirmed in a herd, the following control measure should be considered; isolatation obviously of affected animals to one group if possible, treatment of severely affected animals, affected animals institute measure to minimize public health concern like no raw milk should be consumed) physically clean the environment and disinfection 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 (Huston *e t al*, 2000].

Generally agreement of treatment by supportive therapy and good nursing are important. These include oral or parentral re-hydration, correction of electrolyte balance and stabilization of acid base equilibrium(Venter *et al*, 1994). Calves vaccinated at 1-3 weeks of age with a modified aromatic dependant *S. dublin* bacteria have detectable anti-lipoplisaccharide 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. 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 (Bahnson *et al*, 2006]. The veterinarian research institute produced vaccine against bovine salmonellosis in activated bacteria prepared from isolate of *S. dublin, S. typhomurium* and *S. bovism* or *bificans* and a live attenuated vaccine containing a virulent rough mutant of *S. dublin*(Venter *et al*, 1994)],which will give passive protection to calves for 6 weeks(Huston *et al*, 2002].

Chapter Two

Material and methods

Study area 2-1

Kartoum, the capital of the Sudan,is located in the semi desert zone in between latitude 15.08 and 16.39 North and longitudes 31.36 and 34.25 east and divided in to three major localities (Kartoum,Kartoum North (Bahry) and Omdurman. The topography is flat, expected for some scattered mountains.It is hot and dry with rains in summer and cool and dry in winter,the annual rainfall ranges from 75 to 160 mm, falling mainly in July and August.Generally the dry period extend for 8-10 months.The data from the daily averge minimum temperature is 21.6˚C; the maximum temperature in summer exceeds 40˚C , while the minimum temperature in winter 5˚C. the evaporation (penman) is 7.7 mm/day but during April it reaches 9.3mm/day: the daily average relative humidity is 38% at 8 and 21% at 12 noon .The wind speed is generally about 14.48Km/hour .the population of Khartoum state has grown rapidly in recent years and today is estimated at more than 7 million people, including 2 million refugees from neighboring countries such as Ethiopia and Chad. Intensive live stock production system for milk,meat and poultry are operational within and around Khartoum state.In addition some 1.5 million animal unit pass throw the state for export and trade purposes(El-siddig *et al*,2006).

Study design

2.2Samples collection

From September to October 2012 feces and milk were sampled from farm in Omdurman area (ombada, aboseyde, karary).The farm visited once time of the weak, during five weeks period in random selection of samples, and preserve in ice then to lab. Random samples of 158(100 feces, 58 milk) sterile tubes were collected from cattle in average age from Omdurman (table 1). The samples were preserving in ice and transferred to laboratory and culturing of enrichment solid media.

Table (1) Collection of samples (feces and milk) from Omdurman, Khartoum state

Region	No of farms	Feces	Milk
Abosyede	12	40	20
Ombada	9	30	20
Karari	9	30	18

2.3Microbiology

2.3.1Blood agar

Blood agar prepared by 10 ml of sterile defiriated sheep blood was added to 90 ml nutrient agar which was melted and cooled to 50˚C.The blood agar after mixed well,was distributed (15-20ml) under flame into sterile petridishes and allowed to solidify at room temperature .The prepared plates were kept in the refrigerator (Barrow and felthman,1993).

2.3.2MacConkoney agar

The media was prepared by weighing 48.5 grams of powder disperse in 1 liter of deionized water .Then soaking for 10 minutes ,swirling to mix then sterilized by autoclaving for 15 minutes at 121˚C. C ooling to 47˚C and mixing well befor pouring plates ,prior to inoculation,drying the surface of the agar by partial exposure at 37˚C ,The PH of media 7.4-0.2(Hyde and Denton,2002).

2.3.3Deoxy chocolate agar (DCA)

Deoxy chocolate agar was prepared by weighing 52 grams of powder ,dispersed in 1 liter of deionised water in a two liter flask.Bring to the boiling over gauze,swirling frequently to prevent burning,Simmer for 30 seconds to dissolve.Cooling to 47˚C befor pouring plates.Drying the surface befor inoculation, bile aggregates may appear on the surface on refrigeration,The Ph 7.4-0.2(Hyde and Denton,2002).

2.3.4Nutrient Agar (NA)

The media was parpared by weigh 28 grams of powder ,dispersed in 1 liter of deionised water.Then soak for 10 minutes,swirling to mix and sterilized by autoclaving for 15 minutes at 121˚ C .Cool to 47˚C,mixing well then pour plates.Appearance buff,opalescent gel Ph 7.3-0.2(Hyde and Denton ,2002).

2.3.5Salmonella Shigella Agar (SSA)

Sixty grams of the medium was suspended in one liter of distilled water. Mix well until a homogeneous suspension was obtained. Heating with frequent agitation and boiling for one

minute until complete dissolution. Cooling to 45 – 50°C and the mixture was distributed in Petri dishes. The prepared medium should be stored at 8-15°C. Inoculation and incubation at 35 ± 2°C for 18 – 24 hours. PH 7.4-0.2 The color of the media was red-orange and the colonies yellow black (Pub. Reports. Health, 1950).

2.3.6Xylose lysine Desoxycholate (XLD)

The medium was prepared by Suspended 55.2 g of dehydrated medium (BK058) in 1 liter of distilled water, heating to approximately 90°C with frequent agitation for dissolving .Cooling the medium at 44-47°C,Pour into sterile Petri dishes, until solidied. Incubation of cultured medium at 37°C for 24 to 48 hours, then colonies were appearance pink with black center PH7.4-0.2(Taylor, 1965).

2.4Biochemical test

2.4.1Kliger Iron agar

Fourty nine grams of powder were mixed with 1 liter of distilled water.Bring to the boiling with frequent stirring to dissolveing completely.Then the agar dispense in to tubes and sterilized for15 minutes at 121˚C .Cooling in aslant position over deep butts approx.3cm in depth pH 7.4-0.2(Hyde and Denton,2002).

2.4.2Simon citrate agar

The medium was prepared by weighing 24 grams of powder, dissolved in liter of deionised water, soaking for 10 minutes,swirling to mixed agar, then heating to dissolve the agar and cooling in aslant position.Dispense in to tubes or bottles then sterilize by autoclaving at 121˚c for 15 minutes,and then appearance green, opalescent PH6.9±0.2(Hyde andDenton,2002).

2.4.3Catalase test

A small amount of growth colony was placed onto a clean microscopic slide.
A few drops of H2o2 were added onto the colony, a positive result was the rapid evolution of O2 as evidenced by bubbling. (Hyde andDenton,2002).

2.4.4Oxidase test

Commercial oxidase strips were rubbed with 24 hrs fresh culture of tested organism. A purple color indicated oxidase positive. (Hyde andDenton,2002).

2.4.5 Oxidation fermentation test (O.F)

The powder of medium (9.8 gram) was suspended in aliter of distilled water and then boiling. Addition of sugar in the desired concentration and distributed in tubes.Steriliztion in the autoclave at 121˚C for 15 min , inoculation in two long narrow tubes by deep stab inoculation. One tube was covered with oil layer to induce an anaerobic environment that forces the strain to carry out fermentation (Hugh and Leifson, 1953).

2.4.6 Sugar test

Suger were weigh(0.5 gram) and dissolve trypticase(1 gram), Sodium chloride(0.5 gram), and Phenol red(0.0189mg) in 100 ml distilled water and transfer into conical flasks.Addition of 0.5% to 1% of desired carbohydrate into all flasks.Insertion of inverted Durham tubes into all tubes. Sterilizion at 115^0 C for 15 minutes . Inoculation of the broth with bacterial culture and Incubation of the tubes at 18-24 hours at 37^oC.Yellow color means acid production,while yellow color and presence of small bubbles in the inverted durham tubes means acid and gas production. (Hugh and Leifson, 1953).

Urease test 2.4.7

A slope of urea agar medium(2.1 gram in 95 ml of distilled water) was inoculated with the organism ,then incubated and examined after 24 hours,and daily for 7 days for change in the color of the medium to the pink indicating positive result .While yellow color indicating negative result (Hyde and Denton, 2002).

2.4.8Indol test

Peptone water medium was inoculated with test culture and incubated at 37˚C for 48hours .One ml of Kovacs reagent was run down the side of the tube where no color appeared on the reagent layer within a minute in positive sample(Barrow and fethman,1993).

2.4.9 Motility test

The medium was suspended {22 gram} in one liter of purified water. Heating with frequent agitation and boiling for one minute to complete dissolving. Autoclaving at 121°C for 15 minutes**.**

Stabbing by the colonies were inoculation at the proper temperature and examine at 18 – 48 hours. Diffused growth was spreading from the line of inoculation.. (Tittsler and Sandholzer, 1936).

2.5 Staining

2.5.1Grams stain

A loopful of the liquid culture was transfer to the surface of a clean glass slide,and spread over small area .Tow to four cultures may be stained on the same slide,which can be divided into 2-4 section with vertical red wax pencil lines.For making smear on a slide was placed drop for dilution then small amount of tested culture ,then the film was drying by the air .Fixation after film by passing through the Bunsen flame.

2.5.2The staining technique was used as followed:

-Flooding the slide with crystal violet solution for up to one minute, washed off briefly with tap water (not over 5 seconds).
-Flooding slide with Grams Iodine solution, and allowed to act (as a mordant) for about one minute, then washed off with tap water. Drain.

-Excess water was removed from slide and blot. And then decolorization. Flooding slide with 95%alcohol for 10 seconds and washed off with tap water (Smears that are excessively thick may require longer decolorization).

-Flooding slide with safranin (neutral red) solution and allowing to counter stain for 30 second. Then washed off by tap water. Draining and dryness of all slides and examined under the oil immersion lens. The gram negative organism stained bale pink color.

2.6 Bacteriological count

Label five tubes of saline 10-1, 10-2, 10-3, 10-4and 10-5

-Using aseptic technique, the initial dilution (10-1) is made by transferring 1ml of organism sample to a 99ml sterile saline blank.

The 10-1 dilution is then shaken by grasping the tube between the palms of both hands and rotating quickly to create avotrex.This serves to distribute the bacteria and break any clump.

-Immediately after the 10-1 dilution has been shaken, uncap it and aseptically transfer 1ml to a second 99ml saline blank. This second dilution represents a 10-2 dilution of the original sample.

-Shakeing of the 10-2 dilution vigorously and transfer 1ml to the third 99ml blank. This third dilution represents a 10-3 dilution of the original sample.
-Shakeing the 10-3 dilution vigorously and transfer 1ml to fourth 99ml blank. This forth dilution represents as 10-4 dilution of the original sample. Repeat the process once more to produce a 10-5 dilution.
-Shakeing the 10-4 diluation again and aseptically transfer 1 ml to culture in nutrient agar and 1ml to another nutrient agar.Do the same for the 10-5 dilution.Then incubated at 37˚c for 24 hours.

_Calculation the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor).

2.7Questionnaire design

The data including 20 questions on herd size and type, housing condition, feeding practice, cleaning, health and disease

prevention(Table 2).The questionnaire divided to closed (yes or no /45%) semi closed eg(number of animals, herd composition/30%) and open-ended eg(disease, source of feed/25%). .

Table 2: Collection of questionnaire data in Omdurman locality-Khartoum state.

Subject	Factor
Herd type	Type and number of animals present (cattle,sheep,goat)
Hosing	Herd size, using outside bull, herd mix with others.
Feeding	Mixed in farm,dal,babeker
Cleaning	Weakely,monthly,often
Health and disease prevention	Disease in farm, signs of salmoellosis,age category most affected

2.8Statistical analysis

Data were collected and stored in Microsoft excel and then analyzed by using SPSS version 16.0. All bacteria count were converted to log 10 CFU cm-2 for analysis and ANOVA was performed, Statistical significance was set at statically significant a ($p\_value<0.05$).

Where zeros cause problems with computation of the odds ratio or its standard error, 0.5 is added to all cells (a, b, c, d) (Deeks and Higgins, 2010). Odds Ratio formula (OR) = (a x d)/ (b x c).

P-value formula = OR/ (1+OR)

CI 95% formula = OR± 1.96(SE) $\mathrm{SE}\{\ln(OR)\} = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$

Chapter three

Result

The results showed that 63 (34.04%) out of 158 collected samples were positive(Table 3) for *Salmonella spp*.most of the sample were feces [21.62%] and the highest perecentage was in karari [63.0%],but lowest percentage in Abosyede [27.5%].Where as the highest percentage of milk samples was in karari[44%],but lowest perecentage in abosyede (35%) as shown in(table 4). The study revealed a statistically, no significant difference at P-value ($p \leq 0.05$) between the investigated points. The TVC revealed the highest contamination level was in feces sample (2.00±1.22 log10 cfu/g), while the lowest contamination level was in milk (2.09±0.40 log10 cfu/g).(Table 5)

Table4: prevalence rate of *Salmonella spp* collected from 30 farms in selected locations in Omdurman –Khartoum state.

Sample	Prevalence	Specific Prevalence		
		(Abosyede)	(Ombada)	(Karari)
Feces	40.0%	27.5%	60.0%	63.6%
Milk	39.9%	35.0%	40.0%	44.4%

Table 3: Distribution of isolated *Salmonella spp* from Omdurman location –Khartoum state.

Location	No of Farms	No of samples examined	No of positive samples	Feces	Milk
Abosyede	12	60	18	11	7
Ombada	9	50	26	18	8
Karari	9	48	19	11	8
Total	30	158	63	40	23

Table 5: Comparison of the Mean Total Viable Count of Bacteria (log10 cfu/g) ± SD of Different samples in Omdurman.khartoum state.

Samples	Mean ± SD of TVCs of bacteria (log10 cfu/g)	Significance
Feces	2.00±1.22	NS
Milk	2.09±0.40	NS

Table (6): Demographical characteristics of herds which exposures to salmonella

Variables	Frequency	Percent (%)	Cumulative Frequency (%)
Locality			
Ombada	9	30	30
Karary	9	30	60
Aboseed	12	40	100
Age			
>year	19	63.3	63.3
≤year	11	36.7	100
Sex			
Male	5	16.7	16.7
Female	25	83.3	100
Source of animal			
In side	27	90	90
Out side	3	10	100
Herd size			
<20	4	13.3	13.3
20-100	23	76.7	90
>100	3	10	100

Table 6 showed the frequency of farms which examined and percentage of *Salmonella* infecton according to heard and locality was variable (Ombada f= 9,p=30%, karary f=9,p=30%,aboseyde f=12, p=40%),Age(>year f=19,p=63.3%,≤year f=11 p=36.7%),sex(male f=5 p=16.7%,female f=25 p=83.3%) source of animal(in side f=27 p=90%,outside` f=3 p=10%) Herd size (<20 f=4 p= 13.3%,20-100 f=23 p=76.7%,>100 f=3 p=10%)

Table (7): Univarate chi-square analysis of herd-level risk factors for *Salmonella* in Omdurman.khartoum

Red color explains the risk factors which have significant associations to salmonella at 90% CI.

Variable	Herd Number	(%)	χ^2	Df	P-value
Locality			1.55	2	0.46
Ombada	9	30			
Karary	9	30			
Aboseed	12	40			
Age			0.59	1	0.43
>year	19	63.3			
≤year	11	36.7			
Sex			5.17	1	0.02
Male	5	16.7			
Female	25	83.3			
Source of animal			9.31	1	0.00
In side	27	90			
Out side	3	10			
Herd size			0.31	2	0.85
<20	4	13.3			
20-100	23	76.7			
>100	3	10			
Using outside bulls			9.31	1	0.00
Yes	27	90			
No	3	10			
Mixing with other animals			3.39	1	0.65
Yes	23	76.7			
No	7	23.3			
Source of feed			0.15	1	0.69
In side	4	13.3			
Out side	26	86.7			

House cleaning Often Weekly Monthly	 7 20 3	 23.3 66.7 10	0.51	2	0.77
Other disease Yes No	 23 7	 76.7 23.3	0.31	1	0.57
Season Summer Winter Rainy	 28 2 0	 93.3 6.7	0.07	2	0.78
Abortion Yes No	 8 22	 26.7 73.3	0.37	1	0.54
DVO visiting Yes No	 28 2	 93.3 6.7	0.07	2	0.78
Treatment Yes No	 30 0	 100 0	--	--	--

The chi-square analysis showed significant association between *Salmonella* infection and sex (p-value=0.02) and highly significance between *Salmonella* and source of animal (p-value=0.00) and using outside bulls (p-value=0.00).

Chapter Four

DISCUSSION

The microbial contamination of milk is multi-factorial originating from sources like the air, feed, soil, faeces, grasses and the milking cow itself. Other possible sources of milk contamination include; utensils and water used in the collection and processing of milk (Coorevit *et al.,* 2008). There is little quantitative data available on *Salmonella* present in cattle faeces and such information is important for understanding the impact of faeces on carcasses contamination and for quantitative risk assessments (Cudjoe *et al.,* 1994*;* Cudjoe and Krona, 1997).

In the present study, the prevalence of *Salmonella* in cattle feces tested in this study was 40% which despite differences in methodology is similar to the prevalence of *Salmonella* reported in other studies in European animals has ranged from 1-42% (Williams *et al.,* 1978*;* Vella *and* Cuschieri, 1995*).* In Australia, cattle at slaughter have been studied extensively in the past with reports of Salmonella prevalence in faeces and caecal contents ranging from 57 to 77% (Grau *and* Brownlie, 1968*;* Samuel *et al.,* 1980, 1981). The study revealed an overall prevalence of *Salmonella* in milk (39.9%), which is almost similar to feces prevalence rate. The study result is higher than (11.8%) reported by Karns *et al*. (2005) and the (2.6%) reported by Van Kessel *et al*. (2004) and Mhone, *et al.* (2012) respectively. These variations may be explained by the differences in location,

hygienic practices and the availability of potable water. The quality of agricultural practices observed on the farms might also contributed. This suggests that the milk contamination might have arisen from the lactating cows, themselves, humans or unhygienic milking practices (Addis *et al*., 2011).

Syeda *et al* (2012) had recorded 6% of *Salmonella* in raw milk with average count of log10 0.76 cfu/ml which is lower than our results that may be due to the environment which play an important role in spread of *Salmonella* (fodder water). Raw milk in Italy (Supino *et al*., 2004) had total bacterial counts of 5.23 log cfu/ml and should be due to inadequate sanitary condition during milking, collection and transportation. However, factors other than feeding, such as the breed of the cattle, may have influenced the prevalence results. Contamination of raw milk could originate from surrounding environment especially during milking and milk handling, from water and milking equipment and facilities (Bille *et al*, 2009). Well-constructed herd structure, milking and pre-storage conditions are also determinants of the quality and safety of raw milk (Bonfoh *et al*, 2003). The use of safe/boiled and portable clean water with detergent in washing milking equipment, hands and udder is agood way to remove milk remains including pathogens and, therefore, affecting Themicrobiological safety of raw milk (Chye *et al*, 2004). In general, *Salmonella* prevalence observed inArusha and Arumeru districts was relatively higher

compared to 20% in Ethiopia (Tadesse andDabassa, 2012) and 8.7% in Nigeria. In contrast, the prevalence observed in Arusha and Arumeru districts was relatively lower compared 70% in India (Pant et al., 2013). Still should be investigated for their influence on Salmonella prevalence. This prevalence was clearly higher than the range of 3.34 - 4.0%, reported in the limited studies conducted previously (Soliman and Khan, 1959; Khan, 1962; El Tom et al., 1999). Several studies in other developing countries have reported a higher overall Prevalence of *Salmonella* (human, food, and animal) such as 68.2% in Ethiopia, 51.2% in Argentina , 25.9% in Korea, and 72% in Thailand. It is important to recognize that the prevalence and distribution of *Salmonella* serovars varies from region to region (Dominguez et al., 2002; Uyttendaele et al., 1998) and isolation rates depend upon the country where the study was carried out, the sampling plan and the detection limit of the methodology (Roberts, 1982; Uyttendaele et al., 1998). Consequently, it is difficult to make comparisons between *Salmonella* surveillance conducted in different countries. The CI interval of the farm-level prevalence could be reduced only to a small extent bythe given variables. The risk factors with a significant influence on Salmonella, (e.g., source of animal and using out side bulls).In this result using outside bulls showed highly significant(p-value=0.04, OR = 33),source of animal(p-value=0.04,OR=o) .The mixing with other animal showed no

significance with salmonella but Salmonella prevalence in herds where a cat could enter the pig units is in line with a study by Velinget al. (2002) in dairy herds, those authors ascribed the lower prevalence to the role of cats as predator of rodents and birds carrying or excreting Salmonella. However, Evans and Davies (1996) identified cats as a risk factor (in cattle) because cats are potential carriers.

We found no significant associations with feeding source. In Davies et al. (1997b), meal feed was associated with a lower prevalence of Salmonella compared to pelleted feed. Jørgensenet al. (1999) revealed that coarsely ground feed tended to reduce the Salmonella prevalence. .As generally accepted, one of the tools to control Salmonella is to implement strict cleaning and disinfection protocols, to have hygiene facilities, to practice a strict all-in/all-out policy and to eradicate rodents (Steinbach and Kroell, 1999;Wong Lo Fo et al., 2002). However, none of the investigated hygiene measures was significant in our study.

CONCLUSIONS

Salmonella *spp* are important foodborne pathogenic bacteria that can be harbored sub clinically in the intestinal tract of cattle. The presence of a relative high level of prevalence of *Salmonella* in feces and milk in the study area that reflects the hygienic status in dairy farms. It is suggested that good agricultural practices and adequate hygiene can be employed at the farm level and milk be processed using potable water to ensure public health.

RECOMINDATIONS

11 action steps were suggested to tackle herd Salmonellosis:

1. **Break the fecal-oral transmission link** by minimizing fecal contamination of feedstuffs, feeding surfaces, water troughs and equipment.
2. **Maximize host resistance** of susceptible animals (transition animals and newborns) and **minimize exposure dose**.
3. Control anything in the livestock environment that can perpetuate the organism – rodents, flies, nuisance birds, feral dogs and cats.
4. Because many of the infected animals are subclinical, **in an outbreak, handle all animals as if they were shedding**.
5. Implement a sound **sanitation program based on cleaning all organic matter** – feces, saliva, milk and blood – prior to use of disinfectants (orthophenylphenol on surfaces and boots and chlorhexidine for equipment).
6. Look for development of **newer vaccines** that target signaling pathways and other unique strategies rather than relying on conventional bacterins for prevention and control.
7. A **healthy intestinal environment** gives cattle a competitive resistance. Survival of competitive lactobacilli offer resistance to calves. Maintain the normal gram negative flora (< 1% of the gi mass of bacteria and these are primarily anaerobes) by minimizing oral antibiotic therapy.

8. **Maximize rumen function** by consistent DMI in transition and parturient cows. VFA's are toxic to salmonella.
9. Recognize extended survival time of salmonellae in the environment and deal with potential for spread 4 to 5 years after outbreak.
10. Minimize the chance for salmonellae to replicate by minimizing time in moist, warm environmental conditions. Mix feeds in smaller batches and feed soon after mixing. [Don't feed the waste TMR to young stock].
11. Warn farm families about **zoonotic potential** and assist them in implementing the steps below to minimize the risk.

Avoiding Zoonoses

- ♦ Outer layer of protective clothing is left at farm or at the site of contamination
- ♦ Gloves and protective wear
- ♦ Hand washing
- ♦ No eating or drinking in work areas
- ♦ Don't drink raw milk

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