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OCCURRENCE AND ANTIBIOTIC RESISTANCE OF SALMONELLA SP. IN MUTTON IN WET MARKETS IN SERDANG, SELANGOR

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OCCURRENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA SP*. IN MUTTON IN WET MARKETS IN SERDANG, SELANGOR.

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It is hereby certified that we have read this project paper entitled "Occurrence and Antibiotic Resistance of *Salmonella sp.* in Mutton in Wet Markets in Serdang, Selangor", by Muhamad Faizal bin Hahlan and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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This project is specially dedicated to

MY PARENTS

Hahlan bin Hassan
Noor Liza binti Abu Bakar

MY SIBLINGS

Hazri Hahlan
Quzier Hahlan
Izeham Hahlan

MY CATS

Eba
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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek Ilmiah Tahun Akhir.

KEHADIRAN DAN KERENTANAN ANTIBIOTIK *SALMONELLA SP.* DALAM DAGING KAMBING DI PASAR BASAH SEKITAR KAWASAN SERDANG, SELANGOR.

Oleh

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Salmonella sp. adalah bakteria patogenik utama pada manusia dan juga pada haiwan, yang menyebabkan gastroenteritis akut. Salmonellosis kekal sebagai masalah kesihatan awam yang utama di seluruh dunia terutamanya di negara-negara membangun. Objektif kajian ini adalah untuk menentukan kehadiran dan corak kerentanan antibiotik *Salmonella sp.* yang dipencilkan daripada daging kambing di pasar basah sekitar kawasan Serdang, Selangor. Sebanyak 30 sampel rawak daging kambing telah dikumpulkan dari kedai menjual daging yang berbeza di pasar basah dan kemudiannya diproses untuk pengasingan *Salmonella sp.* dengan cara inokulat

kesemua sampel ke dalam larutan penggayaan Rappaport-Vassiliadis (RV) (Oxoid) dan kemudian ke dalam agar *Xylose lysine deoxycholate* (XLD) dan agar *Briliant Green* (BGA) (Merck). Kesemua sampel didapati negatif untuk *Salmonella* sp.. Kajian ini menunjukkan daging kambing dalam pasaran basah bebas daripada *Salmonella sp*. serta menunjukkan bahawa amalan kebersihan yang baik di rumah sembelih telah dilaksanakan. Kajian berikutnya dengan saiz sampel yang lebih besar dan meliputi seluruh Malaysia diperlukan pada masa akan datang.

Kata kunci: daging kambing, pasar basah, *Salmonella sp*., kerentanan antibiotik

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999-Final Year Project.

OCCURRENCE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA SP.* IN MUTTON IN WET MARKETS IN SERDANG, SELANGOR.

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Salmonella sp. is the major pathogenic bacteria in humans as well as in animals, which causes acute gastroenteritis. Salmonellosis remains an important public health problem worldwide especially in developing countries. The objectives of the study were to determine the occurrence and antibiotic resistance patterns of *Salmonella sp*. isolated from mutton in wet market in Serdang, Selangor. A total of 30 random samples of mutton were collected from different butcher's shops in wet market and processed for isolation of *Salmonella sp.* by inoculating the samples into Rappaport-Vassiliadis (RV) Enrichment Broth (Oxoid) and later into Xylose lysine deoxycholate agar (XLD) and Briliant Green Agar (BGA) (Merck). All samples

were found negative for *Salmonella sp*. This study highlights the mutton in wet market was free from *Salmonella sp.* and indicates that good hygienic practices in slaughterhouse was implemented. Consecutive studies with bigger sample sizes and covering all over Malaysia are warranted in future.

Keywords: mutton, wet market, *Salmonella sp*., antibiotic resistance

1.0 INTRODUCTION

Foodborne diseases caused by microorganisms are the number one food safety concern among consumers and regulatory agencies (Garvani, 1987). Illnesses attributed to foodborne microorganisms often cause severe symptoms affecting the digestive tract as well as other bodily functions, and in some cases, these illnesses may result in death if untreated. *Salmonella sp.* is the major pathogenic bacteria in humans as well as in animals and remains an important public health problem worldwide, particularly in the developing countries (Rotimi *et al.*, 2008). A high incidence of *Salmonella sp.* contamination has been found in many species, such as pigs, cattle, poultry and humans. Industrial food animal production methods and slaughterhouse practices may increase the spread of *Salmonella* among food producing animals.

In primary production, conditions exist which facilitate the spread of bacteria, such as high density of animals. Moreover, in modern slaughterhouses the rapid rate of production keeps the animals in close proximity to each other throughout processing, leading the transfer of bacteria from carcass to carcass (Capita *et al*., 2007).

In addition, antimicrobial resistance pathogens emergence has become serious health hazard in the world. The usage of antimicrobials to prevent and treat the diseases as well as promote growth in farm animals, exposing a large number of animals to frequently sub-therapeutic concentrations (White *et al*.,

2001) and facilitating the development of antimicrobial resistant bacteria that are subsequently transferred to humans through the food chain. Due to the emergence of antimicrobial resistance in *Salmonella sp.*, the treatment towards salmonellosis becomes ineffective (Padungtod *et al.*, 2006).

Several studies have reported on the prevalence of *Salmonella sp.* and its many serotypes in beef, pork and chicken meat, but little research has been performed on the prevalence in mutton. As the consumption of mutton products rise and the possible risk of *Salmonella sp.* infections in the Malaysia increases, it is important to determine the prevalence of this pathogen in mutton.

1.1 OBJECTIVES

The objectives of this study were:

1. To isolate and identify *Salmonella sp*. in mutton isolated from wet market in Serdang, Selangor.
2. To determine the antimicrobial resistance of *Salmonella sp*. isolates.

2.0 LITERATURE REVIEW

2.1 Salmonellosis

Salmonella sp. is a gram-negative, rod-shaped bacterium that lives in the intestinal tracts of infected animals and humans (Jay, Loessner, and Golden, 2005). They are pathogens that pass from the feces of people or animals to other people or other animals (USDA, 2012). When humans or animals ingest contaminated food and water, the bacteria are once again passed through the fecal-oral route perpetuating the cycle. The international spread of *Salmonella* and its illness is often facilitated through the importation and exportation of contaminated goods (Jay, Loessner, and Golden, 2005).

Symptoms for salmonellosis include diarrhea, abdominal cramps, and fever within 8 to 72 hours after the contaminated food was eaten. Additional symptoms may include chills, headache, nausea, and vomiting. These symptoms usually last for 4 to 7 days and many infected individuals recover without seeking medical attention. The problem arises when infections affect infants and young children, the elderly, and people with compromised immune systems. If this occurs, the infection could become life threatening (USDA, 2012).

Treatment for these individuals includes rehydration and possible intravenous fluids. Some antibiotics can be administered if the infection spreads. As for livestock, infection by *Salmonella sp.* can be either asymptomatic (showing no signs

of infection or illness) or pathogenic. Within pathogenic infections, symptoms of Salmonellosis can range in severity from diarrhea and muscle weakness to more critical or acute symptoms such as convulsions or abortion seen in livestock (WHO, 1988). Antibiotics that are commonly used include ampicillin, trimethoprim-sulfamethoxazole, and ciprofloxacin.

2.2 *Salmonella sp*

Salmonella bacteria are heterogeneous group found in the family Enterobacteriaceae that are divided into two species (*S. enterica* and *S. bongori*) and further divided into seven subspecies. Within these subspecies, the bacteria are further categorized into serogroups or serotypes based on somatic or lipopolysaccharide (O) or flagellar (H) antigens (Iwen, 2013). Most of the Salmonella serotypes belong to *S. enterica*, with serogroups A, B (*S. typhimurium* and *S. heidelberg*), C1 (*S. braenderup*), C2 (*S. newport*), D (*S. enteritidis* and *S. gallinarum*), and E (*S. anatum*) strains being the most common, contributing to 99% of reported Salmonellosis infections (Popoff and Le Minor, 1997). Serogroups in other subspecies of *S. enterica*, as well as the majority of serogroups in *S. bongori* are not commonly found in humans (Popoff and Le Minor, 1997).

To identify Salmonella serogroups, scientists use agglutination kits based on the Kauffman-White method containing antisera and control antigens to distinguish groups, A, B, C1, C2, D, E, and the virulence (Vi) antigen that is used to select specifically for group D (Iwen, 2013). Identifying the serogroup becomes important

in that it aids clinicians in providing proper health care to those infected. Serotyping also allows for scientists, health officials, and the public understand Salmonellosis trends and how or where an outbreak or contamination occurs.

2.3 Goat meat (mutton)

Goat meat refers to the meat of the domestic goat (*Capra aegagrushircus*). It is often called Chevon when it is from adults of five to 18 months of age and Cabrito when it is from young animals. In Malaysia, the word “mutton” is often used to describe both goat and lamb meat, although technically the term refers only to sheep meat. As such, statistics on goat and sheep meat are often lumped together under the heading of mutton.

Goat meat with its strong, gamey flavor, is believed to be favored by certain ethnic groups in Malaysia, and is somewhat unpopular among the general populace due to the widely-held misconception that the meat is high in cholesterol and saturated fats. This generally negative perception may be part of the reason for the per capita consumption of mutton remaining stagnant at below 1 kg per annum over the last two decades.

Increased population growth accompanied by urbanization and increasing affluence is likely to increase the demand for high quality animal proteins in Malaysia. This increased demand presents opportunities for entrepreneurs to venture into animal production. In this respect, goat meat could be a nutritious alternative to

other red meat consumption, and its suitability, as an additional income source to small farmers should be explored further.

The goat meat industry in Malaysia has been lagging behind the other meat sub-sectors and the gap between production and consumption has been growing wider. In order to provide policy directions for the goat meat industry, it is pertinent to gather additional information on the factors that influence consumption as well as examine the consumer preference and perceptions towards goat meat. This information will be useful to derive some indications on the market potential for goat meat.

2.4 *Salmonella sp* in nature

Salmonella can be found in nonsymptomatic animals, animal feeds, and even air. The primary habitat is the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects (Jay, 2005). Bacteria are excreted in feces and can be spread in the environment. *Salmonella* has been isolated from water, soil, plant surfaces, animal feces, eggs, raw meats, raw poultry, and raw seafood, among other sources (FDA, 2009).

Research studies have gained insight in the cycle of contamination of *Salmonella* and dissemination patterns. The transmission of *Salmonella sp.* between production animals (pigs and cattle) and wildlife in Denmark was investigated by Skov *et al*. (2008). Samples from birds (n=2,567), rodents (225), insects and other animals (141) that lived in surrounding areas near farms were analyzed. *Salmonella*

was isolated from insects (22.6%), rodents (5.2%), cats and dogs (6.5%), and wild birds (1.5%) living close to the infected herds. Phenotypical and genotypical typing of the isolates suggested that *Salmonella* was transmitted from infected herds of production animals to wildlife; detection of *S. typhimurium* indicated that birds feeding on insects or invertebrates were at a higher risk of infection compared to birds feeding on seeds and grains.

Humans that have an occupational exposure to *Salmonella* might spread the pathogen to their household if they fail to observe good sanitary practices. Rice *et al*. (2003) conducted a study to assess household contamination with *Salmonella enterica* where any of the residents had an occupational exposure. Exposure could take place on cattle farms with known salmonellosis in cattle, a *Salmonella* research laboratory, or a veterinary clinic experiencing an outbreak of salmonellosis. The authors analyzed vacuum cleaner bags and observed 27% (15/55) of samples from households with occupational exposure to *S. enterica* were positive versus 4.2% (1/24) without known exposure. These findings highlight the risk of transmitting contamination to the household, posing a health risk to the residents (i.e. crawling babies on contaminated carpets).

Food animal production environments have been analyzed to elucidate contamination patterns and persistence. Callaway *et al.* (2005) studied the effects of two production systems on *Salmonella* of sows housed indoors in farrowing stalls (n=52) compared to sows housed outdoors (n=52). No differences were detected in *Salmonella* between indoor and outdoor farrowing huts. Interestingly, the authors found some *Salmonella* genotypes persisted within some wallows for over 5 months,

and genetically indistinguishable *Salmonella* isolates were found in multiple wallows. Gotter *et al.* (2012) showed that areas in the indirect environment, including ceilings, aisles and other surfaces, are possible major but often underestimated causes of residual *Salmonella* in swine farms.

2.5 *Salmonella sp*. in food animal

Members of the genus *Salmonella* remain a potential threat to human and animal health. Human outbreaks have been associated with consumption of contaminated animal products (Vella *et al*., 1995). Animal-adapted *Salmonella* serovars can affect the health and productivity of a herd or a flock and contract sharply with healthy carrier animals infected with non-host adapted serovar that may cause salmonellosis in human population. Salmonellosis in food animals including cattle, poultry, swine, and sheep arises from intensive rearing practice, the use of contaminated and medicated feeds and infected or carrier animals (D'aoust, 1989).

At the abattoir, the initial source of contamination is the carrier animal. Transmission at the abattoir occurs by direct contact between carrier and non-carrier animals and also by exposure to contaminated environment. It has been suggested that stress associated with transportation, overcrowding and feed withdrawal experienced by animals before slaughter increases shedding of *Salmonella* (Ekperigin *et al.,* 1998). This effect was demonstrated in a study in which 30% of Salmonella-free pigs became *Salmonella* carriers after a mock transportation to an

abattoir (William *et al*., 1970). Nonetheless, there appear to be no published data that associate feed withdrawal with an increase in *Salmonella* shedding in cattle. During slaughtering operations these carrier animals are able to contaminate the area, the equipment and personnel, and eventually the final products (Smith *et al*., 1994). Other environmental factors such as insects, rodents and wild birds have also been implicated in the infection of herds and contamination of processing areas.

2.6 Antibiotic resistant of *Salmonella sp*

Resistance can be naturally found in an organism or developed by the use and/or misuse of antimicrobial drugs. Antibiotic resistance in *Salmonella* strains is commonly encoded by resistance-plasmids (R-plasmids) that have been acquired as a result of antibiotic selective pressure in human or veterinary medicine (Threlfall *et al.*, 1990). In turn, the majority of R-plasmids have acquired their resistance genes by a mechanism called transposition in which mobile elements of DNA are inserted into non homologous regions of chromosome or plasmid DNA. These elements or transposons can either be acquired from other plasmids in the same strains, from the chromosome, or from plasmids carried by other bacteria in the host organism. Resistance genes can also be acquired from other bacteria in the host organism, either by conjugation in which the resistance gene is transferred from donor to recipient cell via specialized structure of the donor called pilus or by transduction via a carrier such as bacteriophage. Another mechanism by which resistance genes can arise is via spontaneous mutation of the chromosomal genes, again as a result of selective pressure (Espinasse *et al*., 1993).

Although there is a growing need to update information on drug resistance trends in *Salmonella*, antibiotic resistance typing as such is not a satisfactory method for discrimination within the serovars as resistance plasmids and transposons are unstable traits. Nonetheless, antibiotic resistance typing can be performed in conjunction with serotyping, phage typing and plasmid profile analysis for epidemiological purposes. This method has been used in identifying clones of chloramphenicol resistant *S. typhi* belonging to different phage types which caused major outbreaks in Mexico, India and South-east Asia since the 1970s (Mayer *et al*., 1988). It has also been the method of choice along with plasmid profile for differentiation of clones of multiple drug resistant *S. typhimurium* (Threlfall *et al*., 1996).

3.0 MATERIALS AND METHODS

3.1 Collection of samples

A total of 30 random samples of mutton weighing about 10 to 15 grams were collected from different butcher's shops in wet market around Serdang, Selangor. All samples were collected aseptically and kept in sterile plastic before being transported to Bacteriology Laboratory in UPM, Serdang, Selangor for further processed.

3.2 Isolation and identification of *Salmonella sp.*

Mutton samples were processed for isolation and identification of *Salmonella* according to the technique recommended by the International Organization for Standardization (ISO 6579:2002). Briefly, 10 g of each sample was homogenized in 90 ml of buffered peptone water (BPW) (Oxoid, England) using a laboratory blender (Stomacher 400, Seward, England). The suspension was incubated at 37°C for 16 to 20 hours. Later, 0.1 ml of suspension was inoculated into a tube containing 10 ml of Rappaport-Vassiliadis (RV) magnesium chloride-malachite green broth (Oxoid, England) and was incubated at 37°C for18 to 24 hours.

A loopful from the RV enrichment broth was streaked onto brilliant green (BG) agar (Merck, Darmstadt, Germany) and xylose lysine deoxycholate (XLD) agar (Merck, Darmstadt, Germany). Inoculate agar was incubated at 37°C for 18 to 24 hours. Presumptive *Salmonella* colonies (red colonies with black centers) were

then sub-cultured into nutrient agar and incubated at 37°C for 24 hours to obtain pure cultures. Pure cultures were then cultured onto 5% blood agar for biochemical test.

3.3 Biochemical test for *Salmonella sp.*

Biochemical tests for identification of *Salmonella sp.* were listed in Table 1:

Table 1: *Salmonella sp.* identification test

No.	Biochemical test	Method	Result
1	Urease test	Select 3-5 isolated colonies and pick using a loop. Stab into the agar butt and streak on the agar slant. Incubate at 37°C for 24 hours.	No reaction for *Salmonella sp.*
2	Triple Sugar Iron Agar (TSI)	Select 3-5 isolated colonies and pick using a loop. Stab into agar butt and streak on the agar slant. Incubate at 37°C for 24 hours.	*Salmonella sp.* produce alkaline slant, acid butt with hydrogen sulphide production.
3	Citrate test	Select 3-5 isolated colonies and pick using a loop. Streak on the agar slant. Incubate at 37°C for 24 hours.	*Salmonella sp.* will change the green colour of citrate agar to blue.
4	Sulphide Indole Motility (SIM)	Select 3-5 isolated colonies and pick using a loop and streak onto agar slant. Incubate at 37°C for 24 hours. Forindole test, few drops of Kovac's Indole Reagent is added into the test tubes.	*Salmonella sp.* shows increase in turbidity with hydrogen sulphide production. For indole test, no change of colour for the Kovac's Indole Reagent.
5	Slide agglutination test (SAT)	Conducted using polyvalent "O" antiserum A-S. Mix a loopful of bacteria with drop of normal saline on a microscope slide, and place a drop of the polyvalent on the suspension. Hold slide in slight tilt and sway gently back and forth for 1 minute.	For positive reaction, obvious agglutination will be observed.

4.0 RESULTS

4.1 Occurrence of *Salmonella sp*. from mutton samples

Out of 30 samples, none of the samples were positive for *Salmonella sp*.

4.2 Antibiotic susceptibility test

Since there was no *Salmonella sp.* isolated, antibiotic susceptibility test was not done.

4.3 Other bacteria isolated from the samples

A number of bacteria were isolated and identified from the mutton samples. The isolates are *Citrobacterfreundii, Proteus mirabilis, Escherichia coli* and *Klebsiella sp.* the frequency and percentage of the bacteria isolated was listed in Table 2.

Table 2: Frequency and percentage of bacteria isolates from mutton samples

Bacteria	No. of sample with isolates	Percentage (%)
Citrobacterfreundii,	15	50
Proteus mirabilis	8	27
Escherichia coli	4	13
Klebsiella sp.	3	10
TOTAL	30	100

5.0 DISCUSSION

Very little research has been conducted on the prevalence and antibiotic resistance of *Salmonella sp*. from mutton in Malaysia. Studies on different type of meat such as pork, beef, and poultry, as well as studies focusing on different gram-negative bacteria have been conducted and can be useful tool in comparing related research experiments.

Based on the result, it shows that the mutton in Serdang, Selangor are free from *Salmonella sp.*. The result is consistent with the previous studies done by Lim *et al*. (1983) and Molla *et al*. (2006), which reported a low prevalence of *Salmonella sp.* in mutton.

There are a few reasons why there is no occurrence of *Salmonella sp.* in mutton in this present study. First of all, the area of study was limited which is only covered Serdang area, which is a small city in Selangor. Therefore, bigger scope of study was suggested to cover the whole state or country so that the findings to be more meaningful.

Good farm management and biosecurity might also contribute to no occurrence of *Salmonella sp.* in mutton. This is because, due to proper husbandry management starting from the farm, it can control the outbreak of the harmful diseases. Nowadays, most of the farmers alert with the clinical signs shown by the sick goats and they can isolate the infected animals so that the diseases will not

spread to the whole farm. Moreover, most of the farm practices good biosecurity in which they restrict outsiders from coming in to their farm. This is to maintain the 'pathogen free' zone as well as to make sure that the goats in the farm not in stress condition so that the animals can maintain their good status of health.

Therefore, for implication of the study, there is no *Salmonella sp.* isolated from all the mutton samples collected. It means that they are free from Salmonellosis. Since there should be an adverse interaction between agent, host and environment in order to disease to occur, with the absence of the *Salmonella sp.*, which is the agent, disease can't occur.

Usually, *Salmonella sp.* can be found along the intestinal tract of the host itself. In order for *Salmonella sp.* to be on the mutton, there should be physical contact that can transfer the bacteria to the place where it is not the normal flora. According to Capita *et al.* (2007), due to the rapid rate of production that keeps the animals in close proximity to each other throughout processing, it will then lead to the transfer of bacteria from carcass to carcass. Since in this study, there is no occurrence of *Salmonella sp*. in mutton, it can conclude that the slaughterhouses in Selangor practicing good hygienic method in slaughtering the animals. Hazard Analysis and Critical Control Point (HACCP) is one of the systemic preventive approaches at the slaughterhouses that are widely been used as the basic guidelines to make sure the quality of the raw product is safe to eat.

Citrobacter freundii, Proteus mirabilis, Escherichia coli and *Klebsiella sp.* were successfully isolated from mutton in this present study. According to George *et al*. (1968), the incidence of other isolates may be transferred to the carcass while handling and processing the meat. In addition, according to Rao *et al*. (1979), the incidence of *Proteus sp.* in raw meat is fairly high and this is consistent in this study. Although there is no occurrence of *Salmonella sp.* in this study, the presence of other pathogenic bacteria that can cause other infectious diseases should be noted. The findings indicates that hygienic practice was not completely followed by mutton handler or butchers. Proper cooking might reduce the possibilities of getting the infection since most of the pathogenic bacteria can be kill by using high temperature.

6.0 CONCLUSION AND RECOMMENDATIONS

In this present study, *Salmonella sp.* is not present in mutton in wet markets in Serdang, Selangor.

As recommendations, for future study, there are several ways to make the results more significant. First of all, the number of sample size should be increase. With larger number of sample size, it can further increase the chances of getting *Salmonella sp.* in mutton. Next, the sampling area should be extend further by not only focusing on one state, maybe can involve in different city which comprising several state in Malaysia. Using highly sensitive and specific methods in detection of *Salmonella sp.* such as polymerase chain reaction (PCR) can increase the chances in detecting *Salmonella sp.* in mutton. Furthermore, this technique is rapid compared to isolation and identification technique. Lastly, antibiotic sensitivity test should be done to all other isolates. This is important to provide basic information on the current status of antibiotics misused in the treatment of the disease in humans as well as in animals.

7.0 REFERENCES

Callaway, T.R., Keen, J.E., Edrington, T.S., Baumgard, L.H., Spicer, L., Fonda, E.S., Griswold, K.E., et al. (2005). Fecal Prevalence and Diversity of *Salmonella* Species in Lactating Dairy Cattle in Four States. *J Dairy Sci*, 88:3603-8.

Callaway, T.R., Edrington, T.S., Anderson, R.C., Byrd, J.A., and Nisbet, D.J. (2008). Gastrointestinal Microbial Ecology and the Safety of our Food Supply as Related to *Salmonella*. *J Anim Sci*; 43:163–72.

Capita, R., Alonso-Calleja, C., and Prieto, M. (2007). Prevalence of *Salmonella enterica* Serovars and Genovars from Chicken Carcasses in Slaughter Houses in Spain. *Journal of Applied Microbiology*. 103:1366-1375.

D'aoust, J.Y., and Michael P.Doyle. (1989). *Salmonella*. in: Food Borne Bacterial Pathogens. New York and Basel: *Marcel Dekker, INC*, 327-445.

Ekperlgln, H.E., and Nagaraja, W. (1998). Microbial Food Borne Pathogens. *Salmonella. VetClin North Am Food Anim Pract*. 14:17-29. Emergence of Multidrug-Resistant *Salmonella Sp*. and Isolates with Reduced Susceptibility to Ciprofloxacin in Kuwait and The United Arab Emirates. *Diagn Microbiol Infect*, 60:71-7

Espinasse J. (1993). Responsible Use of Antimicrobials in Veterinary Medicine: Perspectives in France. *Vet Microbiol*. 35:289-301.

Garvani, R.B. (1987). Food Science Facts. Dairy Environ. Sanit. 7:20 http://www.journalofanimalscience.org. (Accessed 26 January 2015)

Iwen, P.C. (2013). *Salmonella* Typing. http://www.nphl.org/salmonellatyping-iwen.pdf.pdf (Accessed on 11 January 2015)

Jay, J.M., Loessner, M.J., and Golden, D.A.. (2005). Fact Sheets: Foodborne Illness and Disease. Modern Food Microbiology. Springer Science and Business Media, LLC. 7th Edition. www.usda.gov. (Accessed 12 January 2015)

Lim, Y.S., Khor, S.Y., Jegathesan, M., and Kang, S.H. (1989). The Isolation of *Salmonella* from Raw, Cooked and Dried Foods. *Med. 1. Malaysia Vol. 39 No. 3* : 220-224

Molla, B., and Mesfin, A. (2003). A Survey of *Salmonella* Contamination in Chicken Carcass and Giblets in Central Ethiopia. *Revue Méd. Vét*., 154, 4, 267-270.

Padungtod, P., and Kaneene, J.B. (2006). *Salmonella* in Food Animals and Humans in Northern Thailand. *Int J Food Microbiol.* 108:346-354

Popoff, M.Y., and Le Minor, L., (1997). Taxonomie du genre *Salmonella*. Changements de la nomenclature des sérovars. In: Formules antigéniques des sérovars de *Salmonella*, 7th revision. WHO Collaborating Centre for Reference and Research on *Salmonella*. Institut Pasteur, Paris, France, p. 4.

Rao, N.M., and Nandi, S.C. (1979). *Salmonella* and Other Entero-Bacteriaceae Group of Organisms Associate with Meat, Bone Meat and Legs in India. *Microbiol.*, 16:170

Rotimi,V.O., Jamal, W., Pal, T., Sonnevend, A., Dimitrov, T.S., Albert, M.J. Smith, B.P., Da Roden, L., Thurmond, M.C., Dilling, C.W, Konrad, H., Pelton, J.A, and Picanso, J.P. (1994). Prevalence of *Salmonella* in Cattle and in the Environment on California Dairies. *J Am Vet Med Assoc*. 205:467-471.

Threlfall, E.J., and Frost,J.A. (1990). The Identification, Typing and Fingerprinting of *Salmonella*: Laboratory Aspects and Epidemiological Applications. *J Appl Bacteriol.* 68:5-16.

Threlfall, J.E., Hampton, M.D., Schofield, S.L., Ward, L.R., Frost, J.A., and Rowe, B. (1996). Epidemiological Application of Differentiating Multiresistant *Salmonella typhimurium* DT104 By Plasmid Profile. *Common Dis Rep CDR* Rev. 6:155-9.

Vela, L., and Cuschler, F.P. (1995). *Salmonella* Excretion in Adult Cattle on the Maitese Island of Gozo. *Rev Sci Tech*.14:777-787.

White, D.G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., McDermott, P.F., McDermott, S., Wagner, D.D., and Meng, J. (2001). The Isolation of Antibiotic-Resistant *Salmonella* from Retail Ground Meats. *N Engl J Med* 345:1147-1154.

WHO. (1988). Salmonellosis Control: The Role of Animal and Product Hygiene.

Williams, L.P, and Newell, W. (1970). *Salmonella* Excretion in Joy-Riding Pigs. *Am J Public Health Nations Health.* 60:926-929.