1. INTRODUCTION

The consumption of poultry meat has been increased worldwide within the few years (FAO, 1993; McNamara, 1997; Mead 1997). Competition for an increased share of the poultry meat market centers on lowering the price, thus making poultry more attractive for the consumer. Therefore, modern production system requires a high rate of throughput to meet consumer demand. With complete mechanization, automation, and advanced housing designs (Mead, 1997).

Broilers chickens naturally contain wide range of bacteria, many bacteria are beneficial and necessary for some processes such as food digestion, manufacturing .Classification of bacteria into species is done so disease producing organisms may be separated from those that are harmless or beneficial (Joe .G.Beary 1972;Deibert Whitenack) .Some of harmful bacteria are known for their adverse effects on people and these include as example certain strains of *E.coli* , *Salmonella spp, listeria, streptococcus spp, campylobacter, clostridium spp*. (Hugh Martin ,2002).

Intensive poultry rearing systems have drawn the attention of scientistsfor many years. A number of attempts have been made to introduce newtechnologies in rearing poultry for meat aiming at improvingrearing conditions,protecting the environment and enhancing the quality of poultry products (S.bogosalvjevic, Boskovic, 2011). Environmentally controlled houses with deep litter on the floor, the house temperature, lightening, ventilation must be carefully controlled from day old to slaughter, especially free contaminants feeds and adequate clean water supply can

minimize contamination of broilers chickens (S .Mitrovic , 2011).

The bacterial count in poultry production systems is high in comparison to those of other animals. Little is known about the bacteria present in the poultry environment such as in poultry litter and air of poultry house (Saleh, 2003). Bacteria present in poultry environment may enter into the flock to produce disease. There are also reports indicating that poultry feed and water may act as a source for various infectious diseases, *E. coli* is commonly found in animal feed, indicating that fertilization or contamination with wildlife faeces may potentially be major routes of pathogen transmission (Lynn et al.1998). In addition, poultry itself may act as a reservoir or source of infectious agents for other healthy birds and consequently to the meat processing (Rahman, 1999).

Successful control of bacterial load depends up on the biosecurity system application at the level of the farm and others accompanying control programs, including improvement of entire environmental circumstances and disinfection to prepare healthy chickens submitted to the quality prerequisites (Bolder, 2007).

Food safety was identified as a high priority area in the 2001–2005 World Organisation for Animal Health (OIE) Strategic Plan. Member countries of the OIE considered that the organisation should be more active in issues of public health and consumer protection and that this should include more involvement in the area of diseases or pathogens transmissible through food (Droppers, 2006). Food safety is not only an industry responsibility, but also a major consumer concern. As a result of

society’s heightened awareness about food safety, the poultry industry has recently been faced with producing the same high-quality, cost-efficient product using Hazard Analysis Critical Control Point (HACCP) guidelines (Pope and Cherry, 2000).Meat and meat products are of particular importance regarding food borne illnesses. Food borne pathogens can be introduced to foods during rearing, processing, storage and preparation, from infectedhumans who handle the food or by cross contamination from some other raw agricultural products (Hedberg, MacDonald, &Osterholm, 1994).

Although it is impossible to ensure the complete absence of pathogens from broilers, the risk of food borne disease can be reduced substantially by minimizing their numbers. Mead, (1989) Summarized the reasons why controlling microorganisms in poultry processing is difficult as 1) the rapid rate of production keeps the birds in close proximity throughout processing, 2) limitations in the design of processing equipment, including that used in scalding, defeathering, and evisceration, 3) the difficulty of washing the abdominal cavity effectively after evisceration when the carcass remains whole, 4) retention of water by skin, which tends to entrap bacteria in the crevices and feather follicles (Notermans and Kampelmacher, 1974; McMeekin and Thomas, 1978; Thomas et al., 1987; Lillard, 1989).

Hazard Analysis Critical Control Points is a well accepted systematic program for identification and control of microbiological hazards associated with poultry processing, and has been applied to the poultry industry to improve microbiological quality of broiler carcasses and reduce microbiological hazards from farm to consumption (Unnevehr and Jensen, 1996; McNamara, 1997).

The present study will be carried out to determine which are the points loaded with microbial contaminants pertained to farming remained

undetermined in Sudan, aid the local poultry industry to enhance the quality and safety of their products.

Objectives

General Objective:

The study was aiming to evaluating the potential contamination sources at farm level, including among others, litter, feather, cloaca, transport coops, or free-open range, and breast support track as slaughter house portion by applying principle of selected HACCP.

Specific Objectives:

1. To identify the most contaminated point at the farm level.
2. To determine bacterial number (Total Viable Counts) on broilers on farm.
3. To isolate and identify the contaminating bacteria.

CHAPTER ONE

1. LITERATURE REVIEW

1.1 History of Poultry Industry In the world

Pigeons, ducks, and geese, were bred in china more than 3000 years ago. Chickens, developed from Asian jungle fowl, were domesticated probably about the same time, in ancient and medieval times in the old world; chickens were raised primarily for fighting. In 16 th century, chickens were introduced into America from Europe.

The modern poultry industry began in the late 19 th century in Europe and America as breeders began to stress meat and eggs production .Although eggs were artificially incubated in ancient china and Egypt ,this method of hatching poultry was not used on commercial scale until the 1870`s(Errick,2000).

Discoveries and inventions relating to the scientific housing , feeding ,and breeding of poultry led to the expansion of the industry after 1930`s. Production and consumption of poultry products increased markedly during second world war when meat from other livestock was scarce .Since 1945 ,improved methods of storing and distribution poultry meat and eggs have helped stimulated consumption of these food .Important in the expansion of the poultry industry has been specialized in raising broilers(Errick ,2000).

1.2 History of Poultry Industry in the Sudan

History of the poultry industries in the Sudan began in 1926, by enter a group of Yandotte Chicken from British, followed establishment of the central poultry farm in Khartoum Bahri in 1951 this was starting point of government investment in the field of poultry farming. In 1958 was published a first version of a book on behalf of poultry (poultry farming in the Sudan) to author A. A. Makelmenjeri. Late in 1963 the American Aid Programme established Kuku Poultry Farm. Breeds such as White Leghorn, Fayoumi, Rhode Island Red, New Hampshire and Light Sussex were introduced into the Sudan.

During the period from 2001 - 2005 a significant increase in the number of farms, as a result of growing demand and an improvement in selling prices, especially after the increase in population steady in the state of Khartoum. According to field survey in 2009 the production of broilers was 17.3 million chicks, and the poultry factories in Khartoum state, were about 10 factories of the poultry broilers production with capacity of 25000 tons / hour. Nagla , (1998).

The major source of chicken meat and egg in the Sudan was produced from a population estimated in 1975-1976 to be about 22 million bird yielding 1.3 million kilograms of meat (El-lssawi, 1977). He also stated that the Government was well aware of the importance of developing poultry in the Sudan and hence laid down in 1976 the promotion of Agriculture Investment Act. As a result of this Act a joint Venture the Sudanese Alkwietia Poultry Company was established which at present called the African Poultry Company. The production was five million bird and 40.000 tons of poultry feed. The Arabia Poultry Company started operation in 1984 for Poultry meat and egg self-satisfaction. It produced million eggs and two million kilograms of poultry meat yearly. The broilers produced were inspected pre-slaughtering. A broiler was the trade name used for a young male or

female chicken about 1.5 kilograms and 8 week of age (Gracey, 1981). With the establishment of the broilers processing in Khartoum State, the inspection of poultry meat has been covered in the Meat Inspection Act of the 1974 of the Sudan Laws(Nagla, 1998).

1.3 Poultry meat contribution in people food

Human population growth, urbanization and income improvement are causes of increased demands for food of animals origin in the developing countries (Abdullah et al., 2011; Steinfeld 2003).It is reviewed that shortages of animal protein availability is problem in Africa (Mengesha ,2011) .Based on this demand , there has been raised in the production of foods of animals origin , particularly from poultry in the world. In this regard, (FAO, 2010) reported that contribution of poultry meat is around 33% of the total global meat production. However, this phenomena is not true for undeveloped countries in Africa, rather declining (FOW, 2011, B. Kearny, 2010 ; Speedy ,2003 ; Delgado and Narrod, 2002).

Ethiopia has the highest number of livestock population in Africa (Solomon el al .,2003) .Out of which , poultry production plays an important role in aural livelihood in Ethiopia (Thomas et al., 2009).However , rising demands for these products has led towards high prices (Ayele and Rich ,2010) of poultry products in country. Poultry meat and eggs production in the most environmentally efficient animal protein systems (Mengesha, 2011; Van der sluis, 2007) in the world. However consumption of animal source food has always been low and declining as result of the low production and the continuous growing of population (FAO, 2055; UN, 2005) for sub-Saharan countries.

Pica–ciamarra and otte (2009) reported that poultry has been the fastest growing sector than any animal farming in some of the

developing countries like for instance India .Of the supply and demand maps for animal source foods to 2030, the most dramatic change in projected for poultry meat in South Asia (FAO , 2011 b).

According to FAO (2009) reports, chickens are usually the cheapest of all domestic meat, particularly for sub-Saharan African and south Asian countries. Poultry meat and eggs are highly nutritious, cheapest, without taboos efficient in feed utilization (Mengesha, 2011; Farrel, 2010; FAO, 2010).

Poultry products are preferred by consumers that these products provide food with highly quality protein and low level of fat with desirable acid profiles (FAO; Costa, 2009).

1.4 Overview of World Chicken Meat Production and Poultry Meat Consumption

World chicken meat production quantities have rose from 58.9 million tons in 2000 to 74.2 million tons in 2007,which means more than a quarter percentage (25.9 %) increase during this period (Anonymous, 2009c). Trend for world chicken meat production during 1990-2007 years period (31.87+2.42 in terms of million tons) showed that the average yearly increase was 2.42 million tons. According to 2007 data, U.S, China and Brazil are the major producers of chicken meat with 21.7 %, 14.6 %and 11.9 %, respectively. These three countries represent nearly nearly half of (48.2 %) total World chicken meat production. In spite of its low percentage in total World chicken meat production (1.3 % in 2007), Turkey still ranks in 14th place. The proportion of World chicken meat in total meat production rose from 13.6 % in 1970 to 24.2 % in 2004, while cattle meat decreased from 39.7 % in 1970 to 23.8 % in 2004 (Anonymous, 2006). The protein deficiency stemmed from cattle meat decreases have to some extent been met by increases in chicken meat.

World poultry meat consumption quantities rose from 40.29 million tons in 1990 to 74.77 million tons in 2003, which means the yearly average increase was 2.74 million tons during the period. The per capita poultry meat consumption of U.S was 50 kg in 2003. This figure is comparatively high compared to that of European Countries, which ranged between 9 kg for Netherlands and 30 kg for Spain as well as that of 12kg recorded for Turkey (Anonymous, 2009c). Israel and Brazil are among the highest poultry meat consumption in terms of per capita with 54.3 kg (in 2005) and 33 kg (in 2003) (Anonymous, 2009c).Factors such as consciousness of consumers on the nutritional and health value of chicken meat (Atay et al., 2004;Anonymous, 2004); income level of households (Jimin et al. 2004); the prices of meats substitute for chicken meat (Magdelaine, 2008; Marrision, 2003), population growth increases (Sayılı, 2006); the absence of religious obstacles (Magdelaine, et.al., 2008) and tastiness (Hayman, 2004) may be effective on the increase of poultry consumption.

1.5 Food Safety, Quality and Consumer Protection

The terms food safety and food quality can sometimes be confusing. Food safety refers to all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer. It is not negotiable. Quality includes all other attributes that influence a product’s value to the consumer. This includes negative attributes such as spoilage, contamination with filth, discoloration, off odours and positive attributes such as the origin, colour, flavour, texture and processing method of the food. This distinction between safety and quality has implications for public policy and influences the nature and content of the food control system most suited to meet predetermined national objectives.

1.5.1 Feed stuff control

Food control as a mandatory regulatory activity of enforcement by national or local authorities to provide consumer protection and ensure that all foods during production, handling, storage, processing, and distribution are safe, wholesome and fit for human consumption; conform to safety and quality requirements; and are honestly and accurately labelled as prescribed by law(FAO/WHO,2001).

The foremost responsibility of food control is to enforce the food law(s) protecting the consumer against unsafe, impure and fraudulently presented food by prohibiting the sale of food not of the nature, substance or quality demanded by the purchaser. Confidence in the safety and integrity of the food supply is an important requirement for consumers. Foodborne disease outbreaks involving agents such as *Escherichia coli*, *Salmonella* and chemical contaminants highlight problems with food safety and increase public anxiety that modern farming systems, food processing and marketing do not provide adequate safeguards for public health. Factors which contribute to potential hazards in foods include improper agricultural practices; poor hygiene at all stages of the food chain; lack of preventive controls in food processing and preparation operations; misuse of chemicals; contaminated raw materials, ingredients and water; inadequate or improper storage, etc.

Specific concerns about food hazards have usually focused on:

1\ Microbiological hazards;

2\ Pesticide residues;

3\ Misuse of food additives;

4\ Chemical contaminants, including biological toxins; and

5\ Adulteration.

The list has been further extended to cover genetically modified organisms, allergens, veterinary drugs residues and growth promoting hormones used in the production of animal products.

Consumers expect protection from hazards occurring along the entire food chain, from primary producer through consumer (often described as the farm-to-table continuum). Protection will only occur if all sectors in the chain operate in an integrated way, and food control systems address all stages of this chain (FAO/WHO, 2001).

1.5.2 Quality and Safety of Poultry farm

New challenges in the protection of animal health, food production chain of animal origin, animal welfare and environmental protection require that developing Countries accept the international plan adopted regulations, standards and Procedures as a basis for gaining competitiveness, inclusion and survival in the global market (Uśćebrka, 2006). The criteria for safe food, as well as elements of producer standards are generally in accordance with the legislation. Standards that apply to the finished product are usually defined as a series of internationally recognized standards for product and or as consumer commercial specifications (Oakland, 1993; BSI, 2000; Baines, 2001). Recognized three types of standards: Minimum quality standards, for example, Product specifications; Reference standards, for example: Codex AlimentariusCommission (CAC) or the United Nations Economic Commission for Europe (UNECE) Standards are internationally recognized; Standards of compatibility, for example. ISO standards or QA schemes. In this last type, the current QA standards focused on the external and internal quality attributes, and to external factors include the criteria that determine production standards, standards of animal welfare, environmental protection, health and security and ethical content. Internal quality attributes are those that are directly related to the same

products, such as poultry meat weight, color, size, coverage of the skin. However, the provision of quality in poultry production, as well as the safety of the final product, cannot be achieved without the implementation of the first two types of standards and compliance rules that arise from them (Andersen and Pettersen, 1994). This is, above all, think of the rules good manufacturer practice (GMP) standards and sanitary operating procedures (SSOP) and the good agricultural practice (GAP), risk analysis and critical control point (HACCP) (Usćebrka, 2007).

Good producer and Agriculture practice good practice producer was determined the Commission directive 91/412/EEC of 23 July 1991. And contains Principles and guidelines for good production practice as part of quality assurance which ensures that the appropriate products manufactured and controlled according to quality standards that apply to the use of these products? Institute of Food Science and Technology (IFST) brought in London in 1998. The guidelines for good production practice food and beverages in this guide describe the previously Requested programs that need to be carried out in order to be well designed GMP.For the principles of GMP in the poultry farms, it is necessary to adequately Implement and apply the principles of good agricultural practice (GAP), which Provide quality primary production, for example the production of the one day old Chickens, as the initial link in the chain of poultry production (Uśćebrka, 2008).

Poultry farming is characterized by the production of large amounts of waste material. Accumulation of waste products from poultry production can be a serious problem for the maintenance of ecosystems, and requires the implementation of the system of managing the environment (Sevic, 2009). GAP standards in the practice reduced the

possible negative impacts on the environment and increase the quality and the transience of products on the market (Uščebrka, 2009).

A classical HACCP plan is put in place in a poultry unit, because as a system, poultry production is no different from any other food manufacturing process (Mc Donald, 2005). Raw materials are sourced, purchased, accepted at delivery, prepared, processed, packaged and dispatched. Flow diagrams and all the other accepted mechanisms of a classical HACCP plan are relatively easy to put in place and implement. However, proper CCPs are few in relation to microbial hazards. In practice, there is much evidence that Salmonella, at least, is controllable on poultry farms, when controlled-environment housing is used. The CCPs are the acceptance at delivery of the day-old chicks and feedstuffs, and the supply of drinking water. If the chicks are free from Salmonella on delivery, then good housekeeping, good husbandry, good manufacturing practice, good hygiene practice - call it what you will - will help to keep them free until the day of dispatch. If the feed and water are pathogen-free, then these sources cannot introduce pathogens into the premises orthe birds. If, on the other hand contaminated chicks, feed or water are accepted into the premises, then there is no later CCP on the farm that will either eliminate or reduce to an acceptable level the pathogens contamination (MacDonald, 2005). In order to prevent pollution during the production of defined steps in the cleaning and sanitation, with the aim to achieve sanitary conditions of production. Therefore, the SSOP, in general, documented the steps that must follow in order to ensure adequate cleaning of surfaces and objects that come in contact with the product, as well as other surfaces. These procedures must be sufficiently detailed and specific. All HACCP plans require SSOP to be documented and review in certain intervals and customized if needed. This reassessment may be regular (e.g. Annual), or extraordinary,

but in both cases must be done responsibly and in the scientific basis (Turner, 2002). SSOP can be very simple or very demanding, depending on their final goal. There are two types of SSOP to: preoperative SSOP that are routinely carried out, a day before the start of production and other type of SSOP are operational or working SSOP that describe routine sanitary procedures are performed during the work process (Kinaki , 2008).

1.6 impact of Biosecurity in poultry farm

Biosecurity can be defined as the actions taken to both reduce the risk of infection entering the farm and to remove infection from the farm (Francine; Bradely, 2000), Plays an important role in minimizing the risks to human health from contaminated poultry meat. Biosecurity is about far more than just controlling food poisoning organisms though; good biosecurity will also improve the performance of the flock (Bolder, 2006).

Food borne free poultry meat production starts on the farm, where biosecurity plays a major role in the control of infection and contamination. Every single part of the production system should be aware of its potential contribution in this respect. There are wide variety of interventions and control measures available to limit infection opportunities. At farm level, a number of interventions or preventive actions can be taken in order to control or eliminate food borne pathogens (Bolder, 2006). These not only apply to growing broilers, but also to the preceding stages of poultry meat production and include hatcheries. At grandparents and parents level vaccination against infected diseases like Salmonella, high standards of biosecurity on farm (Bolder, 2006), and regular monitoring in combination with other control measures are examples of such interventions. Broiler farmers should aim to start with pathogen free premises and bought-in livestock should be

pathogen free as well. It is now feasible to produce broilers without Salmonella, although Campylobacter-free production is still not possible, as even in so called high containment facilities it appears to be difficult to keep pathogens out(Bolder,2006).

Manure management needs attention in poultry production. Manure has to be removed immediately after removal of confirmed Salmonella-contaminated animals to slaughter (Bolder, 2006). Manure cannot be applied on land where crops used for poultry feed are grown. Salmonella could survive for up to one month in litter applied to arable land, and survive for up to 3 months in slurry (Nicholson, 2005).

1.7. Application of biosecurity

Biosecurity involves using all measures possible to control the spread of disease-causing organisms. An all-in-all-out programme followed by a rigorous cleaning and disinfection programme is recommended. Rodent control, especially while the premises are empty, is very important to prevent recontamination of the environment by *Salmonella*-infected rodents after cleaning and disinfection (). The restricted movement of birds, people and equipment, combined with good sanitation, helps to control the spread of disease-causing micro-organisms, and possibly zoonotic pathogens. An important element in disease prevention is to avoid contact between poultry and migratory wild birds and to ensure that small birds cannot again access to the poultry house. As biosecurity on farms is reinforced, the importance of factors such as 'catching crews' must be addressed. Vehicles, cages and crates used for broiler movement can also transmit diseases from farm to farm and can be an important source of Salmonella contamination. Better designed crates and improved ways to sanitize transport crates are critical needs for the industry (Rigby; Pettit; Baker; Bentley, 1980).

2.7.1 Sanitation practices to prevent contamination of the farm

General sanitary guidelines are recommended to minimize contamination on the farm and during transportation(UK ACMSF, 2004). These include: Clean housing and equipment between flocks including disinfection of water supply system, remove all manure between flocks; Minimize exposure of equipment, feed, and flock to wild animals (birds, rodents, etc.), restrict and minimize traffic onto the farm, into the houses, and between flocks, Utilize vehicle and personnel sanitation stations change foot ware, boot dip disinfection, Clean and disinfect transport crates and vehicles after every use. Decontamination treatments should be validated to be effective. High-pressure jet spray treatments on poultry transport containers were ineffective in reducing *coliform* populations. In contrast, both immersion in hot water (60 or 70°C for 0.5 min)or in sodium hypochlorite solutions (1,000 ppm for 2 min)reduced coliforms by an average of 1.6 and 4.2 logs, respectively(Ramesh ,2004).

2.7.2 Workers hygiene

Wearing protective clothing, house dedicated footwear, and/or dipping boots, Washing or sanitizing hands, cleaning and disinfecting the house and any equipment entering that house, Controlling visitors and their equipment and vehicles both to the house and the farm could minimized contamination by pathogens (Allen and Newell, 2005).*Campylobacter* can potentially be carried into broiler houses via boots, external clothes and equipment and biosecurity measures such as an ante-room and shed-specific boots and clothing or a disinfectant boot dip appear to be important.

1.8 Production factors management influencing poultry farming and meat quality

With the initiation of compulsory inspection ,many producers and processors found to their dismay that anew cost for handling and

processing poultry had been added ,that of condemnation .The majority of condemnation are largely results of pathological conditions ,which in turn are influenced to great extent by management practices .The management practices are related to highly condemnation rates and that combination of poor management and adverse weather conditions lead to even higher condemnation (Shmittle, 1961).

1.8.1 Housing and stocking density

Conventional system of production of poultry meat, based on high growth rate and efficient use of feed of broiler hybrids housed in high stocking density, in closed facilities with artificial light and air ventilation, is subject to constant criticism (Sundrum, 2001). In general, interest for the quality of poultry meat is considerably increasing compared with the quantitative aspect of this production. Despite the relatively low price of poultry meat deriving from intensive production, consumers have realized the importance of products from non-industrial systems which ensure not only welfare conditions for chickens, but also provide high nutritional level and bio-safety of the product(Fairchild, 2005).

The modern broiler house enables producers to have great control over the house environment. Birds can be placed at higher densities as long as the correct environment (temperature, ventilation, humidity) is provided. Factors to consider when determining stocking density include but are not limited to bird size, feeder space, drinker space, house dimensions, bird welfare, nutrition, breed, performance and economic return (Boyd, 2001). The ultimate goal is to maximize meat yield of chicken while preventing production losses due to overcrowding. In many cases, producers have to settle for slightly reduced performance to achieve a satisfactory economic return. Another concern with increased stocking density is broiler welfare. Animal activist groups request that

broilers be given more space during grow-out and cite behavioral and physiological stress as the reason (Fairchild, 2005). Increasing stocking density of broilers is a management practice used for reducing costs associated with labor, housing, fuel and equipments. However, crowding of broilers can lead to reductions in performance (Shanawany, 1988). Maintaining a high stocking density is a common practice for the poultry industry because it allows for an increase in economic returns per unit of floor space. However, income per bird often decreases primarily due to reduction in growth rate, increased proportion of downgraded carcasses, and greater risk of health-related problems (Estevez, 1999).

One of the most important consequences of increasing density is the environmental change that occurs within the chicken house. Increase intensity usually results in corresponding increases in temperature, humidity, CO2, and ammonia levels. High ammonia levels over 25 to 50 ppm reduce growth and increase the incidence of air sac inflammation. High humidity and moist litter increase the incidence of breast blisters, hock burns, and foot pad dermatitis (among other effects). However, the magnitude of the effect of density depends on technical factors (e.g., quality of ventilation and cooling systems) as well as management factors (e.g., litter condition and light programs). This means that increasing the number of birds in a well-prepared house can cause fewer negative effects than an increase in a similar incrementing an out-of-date building with poor technical conditions (Estevez, 1999).

consumers awareness of animal welfare and associated changes in the perception of the quality of the product(Sundrum, 2001) and regulations, European (Council Directive 2007/43/EC) and national require certain changes in conditions and management of broiler production which among other things, relate to stocking density and duration of photo period. Numerous researches carried out in order to

determine the optimum stocking density in intensive broiler production from the aspect of production performance and carcass quality (Lewis et al, 1997; Edriss et al., 2003; Mendes et al., 2002; Dozier et al., 2005) but also stress indicators (Dozier et al., 2006; Thaxton et al., 2006; Ozbey and Esen, 2007), indicate the growing importance of this factor. The effect of stocking density on growth rate is direct and associated with the possibility of undisturbed emission of heat generated in the process of nutrient metabolism (Yadgari et al., 2006). Heat sensitivity increases in higher stocking density due to increase in temperature of the litter and limited air circulation around chickens. In addition to this direct impact, stocking density also indirectly influences the microclimate in the facility (ammonia production, litter humidity) and forming of other environment factors of importance for demonstration of high genetic potential of broilers. Continuous light regimes such as 24 L or 23 L: 1D have been a norm in the poultry production for a long time, based on fact that, chickens consume feed only during photo period, so in this way optimal growth intensity was ensured, that is, digestion and absorption of nutrients. However, because of welfare indicators, application of continuous light regimes has been reduced, but it still cannot be established with certain level of reliability which is the optimal duration of photo period within 24 h. According to (Petersen ,2004), broilers must have at least 8 h of dark during 24 h, although, it is anticipated that this period of dark will be reduced to 3 h. According to current Rulebook on Animal Welfare Conditions (2010) fattening chickens, after 7days, must have minimum of 6 h of darkness during 24h Period, with continuous 4 h dark period. Previous researches were focused on application of different types of light regimes, restrictive, discontinuous, with prolonged photo period and their effect on production parameters and incidence of skeletal diseases (Buyse et al., 1996; Gordon, 1999; Sanotra et al., 2002; Guler

and Yalcin, 2004). Only few studies focused on carcass quality traits under the influence of light regimes (Renden et al., 1996; Lien et al., 2007).

House temperature, humidity, and environment interrelated it's difficult to determine the effect a specific variable in the quality of carcasses produced (George; Mountney, 1961). Broilers are housed to prevent abnormally high or low temperature be21.1 C at ultimate age, and 29 to 34 C at brooding, is generally considered the most desirable for broilers. (Muriel, 2003). Found the significance of differences in major meat quality traits (percent yield of primal cuts, tissue percentage in major primal cuts, and chemical composition of muscle tissue) in broilers reared under intensive and semi-intensive systems.

The effect of rearing system on growth and meat quality of broilers hasbeen studied by a number of authors (Lewis, 1997; Ristić, 1999; Damme and Rychlik, 2001; Holcman, 2003; Bogosavljević-Bošković et al., 2006a, 2010) Results of previous research (Bogosavljević-Bošković et al., 2006b, 2011) have shown that broiler hybrids most commonly reared in intensive purposes can be successfully reared under semi-intensive i.e. free-range conditions. Certain meat quality traits obtained under this rearing system have proved to be better as compared to those of intensively reared broilers.Flock size. Larger flocks are associated with increased risk of Campylobacter colonization (Barrios, 2006; Berndtson, 1996) and the effect was independent of bird density.

1.8.2 Ventilation

Air movement helps to control temperature and humidity inside poultry house by removing heat and moisture from the house. Ventilation also helps to remove dust and fumes ,such as carbon dioxide ,carbon

monoxide , and ammonia(Clarks , 1961).Temperature , humidity , and the rates and amount of ventilation play an important role on the comfort of the broilers , because they were considered as main causes of stress .In adequate ridge ventilators , house longer than 150 f, over 35 f , all these housing conditions were found to be correlated with high condemnation of broilers in the plant(Clarks , 1961) .Wide ,and houses located in low area , chickens condemnation were higher than those in high area and narrow houses and solid portions between pens (Cover ,1981).

1.8.3 Insulation

Prevent the loss of warm air in winter and cool air in summer and helps to prevent wide fluctuations in temperature .Because the amount of moisture retained by under a given set of conditions is influenced considerably by temperature, insulation help to keep the inside of poultry house dry, as a result of insulation the air is warmer and retained more moisture than cold air which causes moisture than cold air which causes moisture to condense inside the house (Clarks, 1981).

1.8.4 Litter

Litter absorb moisture when condition in poultry house are damp, released it during dry period. It also absorbs some gases and released them when damp condition occur and provides a good medium for protection and growth of pathogenic organisms especially when damp .Litter is a source of dust and irritation of for growing birds when it becomes too dry ;when too wet it is cold , damp and uncomfortable and add an additional stress. Coarse, damp litter is also one cause of breast blisters in growing broilers and consequently increasing condemnation in plant (Clarks, 1961). To control the microbial load in poultry, keeping the litter dry so carcasses stay visibly clean (Bolder, 2006).

The presence of bacteria in poultry litter may contribute to contaminated processed carcasses by

increasing the bacterial load of skin and feathers or by providing a source for upper gastrointestinal contamination during preharvest feed withdrawal (Bennett, 2003).

The quality of the in-house environment is highly dependent upon litter quality. The litter environment is ideal for bacterial proliferation and ammonia production. The two factors that influence litter conditions most are manure and moisture. The manure portion is largely out of a grower's control; however, growers can and must control litter moisture (Ritz, 2005).

1.8.4.1 Litter bacterial load

Broiler litter is a mixture of a substrate with the feces of birds where many undesirable bacteria may develop, such as *Salmonella spp., Campylobacter spp*. *Clostridium perfringens*, and *Staphylococcus aureus*. The accumulation of these pathogens raises concerns about the flock itself and, especially, about consumer health (1968; Bang et al., Lu et al., 2003; Alexander et al., 2002; Dhanarani et al., 2009; Graham et al., 2009; Terzich et al., 2000; Vizzier-Thaxton et al., 2003).

Isolation done by (Halbrook, 1951) determined that broilers litter was populated with *enterococci*, *lactobacilli* and *coliforms*. Another investigation by (Lovett, 1971) evaluated the bacterial populations in poultry litter. Results indicated that *Escherichia coli* and *coliforms* were a constant inhabitant of the litter. Others have evaluated the microbial population of litter through molecular techniques. Several bacterial species have been identified in broiler litter by (Lu, 2003) using 16s rRNA and functional gene markers. Some ofthe identified bacteria include: *Clostridium*, *Corynebacterium*, *Denitrobacter, Globicatella,*

Staphylococci and *Bordetalla* (Lu, 2003). aerobes, anaerobes and coliforms were highest in the top layer of litter and decreased with increasing litter depth(Barker;Davis,2010).

1.8.5 Diseases control

Because of diseases cause the greatest loss of carcasses during processing (schmittle ,1961) ,that the flock with high condemnation rates did not have satisfactory diseases control programme .Respiratory diseases are the most important factor related to broilers condemnation (schimttle ,1961).Diseases control take place by implementing biosecurity ,vaccination program, and medication and often used these three in varies combination(Yoni segal ,2005).

1.8.6 Interval period or vacant houses

The interval period between successive cycles of breeding or houses not disinfected properly and less than one week at least may cause in stressed broilers and increase susceptibility to diseases at time of harvest (Edgar et al, 1961).

1.8.7 Brooding

The brooding period is a critical time in the life of poultry, and this determines largely the success of the enterprise in general, and broilers management in particular. When broiler chicks are subjected to poor brooding environment during the first two weeks of life, reduced growth rate, increased feed conversion ratio, that follows may not be fully regained before market age (Akrum Hamdy, 2003). Immunosuppression and increased incidence of ascites caused by E.coli , and have been associated with exposure of chicks to low brooding temperature and high ammonia concentrations in the brooder pen(Edgar , 1991).Abnormally high or low temperature and humidity or rapid

changes in temperature or humidity causes stress on live broilers which make them less resistant to diseases (George ,1961) .Such extremes of temperature and humidity also set up condition with in a poultry house which cause further stress situations (Schmittle ,1961).

1.9 Sources of contamination of poultry farm

The main sources of grange site contamination seem to be contaminated feed, horizontal transmission, animals kept in an infected environment, and vectors such as, rodents, insects, wild birds, pets and humans (Baker, 1987).

Although horizontal transmission is well known, the role of vertical transmission continues to be discussed as an epidemiologically important way of fowl infection (Fonseca, 2006).

2.9.1 Feedstuff

Feed has to be safe, both from a chemical and bacteriological point of view. Feed components and raw materials can be vectors for transmission of zoonotic agents such as Salmonella (Jones and Richardson, 2004.). Animal feeds are thought to be an important source of these bacteria**.** Poultry feed components of plants and animal origin are commonly contaminated with microorganisms, mostly bacteria , fungi, and or insects (D'Mello,2006).however ,the number and type of microorganisms vary depending on the functions of materials ,location of its origin ,climatic conditions encountered , harvesting ,processing , storage , transport technologies employed and packaging materials(D'Mello,2006).The impart of the general environmental and handing circumstances including the nature of extent of quality control measures on the level of microbial contamination (Dhand ,1998).Some beneficial poultry feed contaminants such as *lactic acid* bacteria have been reported (Dhand;and D'Mello,1998) .

When bacteria pathogenic either to animal or human hosts contaminates feed, it becomes potential route of transmission of disease to both populations, and consequently of great concern to producers and consumers (Crump, 2002) as with natural bacterial contaminants of feed, soil is the primary vector of inoculation. Soil mixed with animal faeces can contaminate standing crops either by direct deposition or when used as fertilizer (Maciorowski, 2004).

The use of pelleted feed is recommended since the pelleting process generates heat which can kill Salmonella. Dedicated feed delivery vehicles and good sanitation practices as well as biosecurity help prevent poultry feed from being contaminated .Mashed feed should not contain animal protein or should include only animal protein products produced under the animal Protein Producers Industry Salmonella Reduction Education Program (Franco,1994).

1.9.1.1 Origins and survival of pathogens in feed

When bacteria pathogenic either to animal or human hosts contaminate feed, it becomes a potential route of transmission of disease to both populations, and consequently of great concern to producers and consumers (Crump et al., 2002), as with natural bacterial contaminants of feed, soil is the primary vector of inoculation. Soil mixed with animal faeces can contaminate standing crops either by direct deposition or when used as fertilizer (Maciorowskiet al., 2004). Pathogenic bacteria found in fecal material are adapted to a markedly different environment than found in soil. The animal intestine typically is characterized by a low oxidation–reduction potential, sufficiently greater moisture and nutrient concentration than soil. However, the intestinal environment is also highly competitive with limited residence time for potential bacterial inhabitants. Unattached bacteria or bacteria that are sloughed off with mucosal cells leave the intestinal environment and mix with soil bacteria.

A portion of this population must then survive in the relatively desiccated and nutrient poor environment until it may colonize another host. If the surviving bacteria is commensal inhabitant of the gut, such as nonpathogenic E. coli, their contribution to feed microflora may be of marginal concern.

The mechanisms and maximum times of survival of intestinal and pathogenic bacteria outside their host are not completely understood. In a study involving air dried fecal material. *Coli* could be isolated after 85 days of composting, whereas *Salmonella* spp. could not be isolated after a maximum of 25 days (Dorn and Schleiff, 1997). In contrast, Temple et al. (1980) could consistently detect at least 103 E. coli and S. ser. *Typhimurium* in faeces for 8 weeks after shallow burial in soil. Some anaerobic spore-bearing bacteria, such as

Clostridium spp., are able to exist as either vegetative cells or spores to become equally proficient in surviving in both soil and gastrointestinal tracts (Haagsma, 1991).

Excrement from wild or domestic animals feeding on crops or on nearby garbage can be a source of bacterial contamination in crops. In numerous studies, Salmonella spp. have been isolated from mice, rats, possums, skunks, raccoons, pigeons, and crows (Maciorowski et al., 2004; Crump et al., 2002; Tauxe, 2002). Predators, such as foxes and domestic cats, can consume contaminated prey such as mice and insects and become vectors of contamination themselves. Houseflies and cockroaches feeding on fecal matter can act as both vectors and reservoirs for pathogens in the environment (Maciorowski et al., 2004).

1.10 Poultry feeds microbiology

Poultry feeds microbiology has re-emerged as an important issue in the wake of the Salmonella, *E. coli*. Many species of *Salmonella* have been implicated in diseases of farm animals. Of these, *S. typhimurium* is

universally distributed while *S. enteriditis* has emerged as a regular pathogen of poultry (Jeffrey, 1998). Meat and bone meal and fishmeal are frequently contaminated with *Salmonellas* (Lynn, 1998).

(Herron ,1993) determined that *Erwiniaherbicola* and *Rahnellaaquitilis* were the dominant bacteria found on unharvestedgrasses, with lesser numbers of *Serratiafonticola, Enterobacter cloacae, Hafniaalvei*, and *Escherichia coli*. Lin. (1992) noted that, in fresh alfalfa with moisture content of 450 g/kg, *enterobacteriaceae* outnumbered yeasts and moulds. Pelhate. (1988) enumerated microbial populations in wheat containing 180 g moisture/grand reported that, during the first 3 months of storage, bacteria of the genera *Erwina,Enterobacter* and *Pseudomonas* predominated. However, as storage continued and moisture levels fell, microbial numbers decreased. Furuta, (1980) detected no aerobic bacteria could be detected in mineral supplements such as calcium or dicalcium carbonate, which contain virtually no free water. Bacterial populations also vary by feed type. Bacterial populations on grains and seedscan be divided into three different orders. *Pseudomonas* and *Acetobacter* spp. from the order*Pseuomonodales* and *Streptomyces* of the order *Actinomycetale*s have been isolated fromfeed (Richard Molard, 1988). The most diverse order on feeds, and most predominant in astudy by (Lin, 1992), is the order *Eubacteriales*. Bacteria from at least five different.

Eubacteriales families have been reported in a review by (Richard Molard1988), including*Achromobacteriaceae* (*Alcaligenes, Achromobacter*, and *Flavobacterium*), *Enterobacteriaceae (Escherichia, Enterobacter, Paracolobactrum, Proteus, and Serratia), Micrococcaceae (Micrococcus* and *Sarcina*), *Brevibacterium* from *Brevibacteriaceae*, and *Bacillaceae (Bacillus*, Bacterium, and *Clostridium*). *Enterobacteria*

represent a major portion of themicro flora adhering to standing grasses. The numbers of *enterobacteria* on unharvestedgrain grass to be approximately 104 CFU/g. (McCapes, 1989) isolated E. *coli* from unprocessed poultry feed. Each feed type may possess only a few members of order *Eubacteriales*. Detected *Pseudomonas*in oat but not in wheat meal and *Micrococcus spp*. only in wheat meal (Hanis, 1988), Genera that were common across substrates included *Bacillus, Serratia, Clostridium*, and *moulds*.The route of contamination appears to be direct contact with contaminated faeces by *campylobacter ssp* to feed (Hingely, 2000). Oilseed meal and animal derived protein are the major risk feed materials for introducing *Salmonella* contamination to feed mills and industrial compound feed (Fournier, 2001).Microbial populations present on grain and feed products, like those from soil, are also diverse and dependent upon the moisture content of feed.

Analyses of commercially manufactured feeds confirmed that both feed ingredients and dust can be sources of Salmonella contamination in feed mills (Jones and Richardson, 2004). Moreover, some pathogens such as Salmonella species can survive for long periods of time in feed of low water activity (e.g. 16 mos at 25°C and51% relative humidity (Williams and Benson, 1978).

Most of the bacterial isolates are highly pathogenic in poultry feeds implicated *Aerobacteraerogenes ,Bacillus cereus, Micrococcus luteus , Enterococcus faecalis ,Staphylococcus aureus and Listeria spp* (WHO,1992;Klinger and Lapidot,1993;Dhand et al .,1998;Hancock et al .,1998;Jeffry et al.,1998;Dhand et al .,1998;D'Mello,2006).

Cooking and pelleting of poultry and swine feeds generally involves temperatures between 70 and 90°C. At temperatures exceeding 83°C, Salmonella was eliminated during pelleting (Cox, 1986).

Consequently, a decreased risk was associated with feeding chickens pelleted feed compared with feed meal (Rose, 1999).

1.10.1 Supplementation with prebiotics

Lactic acid bacteria and certain other microorganisms can increase resistance to infection and that lactic acid bacteria can be enriched in the intestinal tract by feeding specific prebiotic carbohydrates (Patterson and Burkholder, 2003). Prebiotics are “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health” (Gibson and Roberfroid,1995). An example is lactose because chickens are unable to enzymatically digest lactose. Inclusion of lactose in the diets of chickens reduced cecal colonization of *S. Typhimurium* and was dependent on the inability of the pathogen to ferment lactose (Ziprin et al., 1991). Inhibition was correlated with changes in cecal pH and increases in dissociated volatile fatty acids, especially propionic acid. Other potential prebiotics for reducing colonization of *Salmonella* in chickens include fructooligosaccharide (Fukataet al., 1999), mannose-oligosaccharide (Fernandez et al., 2002), and isomaltooligosaccharide (Chung and Day, 2004). An increased prevalence of *Bifidobacterium* species and *Lactobacillus* species is generally associated with these prebiotics in reducing *Salmonella* colonization (Fernandez, 2002; Xu, 2003).

1.10.2 Water

The World Health Organization (WHO, 1996) guidelines present contaminant levels in drinking water. Specific guidelines are presented for permissible concentrations of (a) bacteria, viruses, and parasites; (b) chemicals of health significance including specific inorganic and organic constituents, pesticides, disinfectants, and disinfection byproducts; (c) radioactive constituents; and (d) substances

and parameters in drinking water that may give rise to complaints from consumers (American Water Work Association [AWWA], 2002) The term "safe" drinking water does not mean risk free; it simply means risks are very small, at or below our ability to quantify them, or that water quality limits cannot be lowered further by economical water treatment processes (Szewzy, 2000) WHO states in 1997 that the infectious diseases caused by pathogenic bacteria, viruses and protozoa or by parasites are the most common and widespread health risk associated with drinking water. Microorganisms transmitted in water generally grow in the intestines and leave the body with faeces (AL-Kahah, 2000).Use good quality clean water (tap or deep wells) treated, sediment, filtrated, and chlorinated water to minimizing water pathogens like *coliform* bacteria (Yoni segal,2005). An intervention programme of chlorination of the water supply, cleaning and disinfection of the drinking system of the grow-out house and withdrawal of furazolidine from feed reduced the proportion of broiler chickens colonised with *Campylobacter jejuni* from 8 1% to 7% and reduced the *C. jejuni* recovered from broiler chicken carcasses by a factor of 1,000 to 10,000(Stern; Jones; Wesley; Rollins, 1994).

1.10.2.1 Water Bacteriology

(WHO, 1997; CDC; Microbe world, 2002) obtained that the main pathogenic bacteria of water were (*Salmonella, campylobacter, Shigella,Vibrio cholerae*, *E.coli,*Yersinia, *Pseudomonas aeruginosa).*A British study showed the link between the presence of *C. jejuni* in drinking water and colonization of broilers (Pearson, 1993). Water was shown to be the predominant source of *Campylobacter jejuni* on a broiler chicken farm. *C. jejuni* was found at all levels of the bore-hole as well as in the sediment at the bottom of the well from which water was obtained for the grow-out house (Rollins; Colwell, 1986). Running or standing

environmental water (rivers, streams, ponds, etc) can be sources of *Campylobacter* species (Kemp, 2005).

The predominant intestinal enterococci are *E. fecalis, E. faecium, E. durans* and *E. hirae*. In addition, other *enterococcus* species and some species of streptococcus (namely *S. bovis*, and *S. equines*) may occasionally be detected. Generally, for water examination purposes *Enterococcai* can be regarded as indicators of fecal contamination, although some can occasionally originate from other habitats (Pinito, 1999).

More recent research on the relevance of fecal *streptococci* as indicators of contamination showed that the majority of *Enterococci* (84%) isolated from a variety of contamination water sources were true fecal species (Pinto, 1999).

1.10.2.2 Drinking Water Treatment

The treatment and distribution of water for safe use is one of the greatest achievements of the twentieth century (AWWA, 2002).

Meeting the goal of clean, safe drinking water requires a multi-barrier approach that includes: (AWWA, 2002).

1. Protecting the source from contamination.

2. Appropriately treating raw water.

3. Ensuring safe distribution of treated water.

The water treatment process involves a series of different steps. Some of the major steps include: (CDC, 2000).

1-Flocculation and coagulation (the joining of small particles of matter in the water into larger ones that can more readily be removed);

2-Sedimentation (the settling of suspended particles in the water to the bottom of basins from which they can be removed);

3-Filtration (the filtering or straining of the water through various types of materials to remove much of the remaining suspended particles);

4-Chemical disinfection:

Chlorination can be defined as the adding of chlorine to water in order to kill any dangerous bacteria that might be present (Daud, 2001)

During the treatment process, chlorine is added to drinking water as elemental chlorine (chlorine gas), sodium hypochlorite solution or dry calcium hypochlorite. When applied to water, each of these forms produce “free chlorine,” which destroys pathogenic (disease-causing) organisms (AWWA, 2002).

1.10.2.3 Elimination of pathogens from water

Many chemical treatments have been evaluated to control pathogens in water sources for livestock and poultry. Operations, treatments such as acidification of water or addition of chemicals (chlorine, ozone, sodium chlorate) have had limited success due to the continuing presence of rumen content or manure in water troughs (Callaway et al., 2002). Consequently, frequent cleaning of troughs is needed to maximize those water disinfectant treatments. Other chemical treatments, on the other hand, can be effective even in the presence of manure or faeces content. For example, treatment combinations containing0.1% lactic acid, 0.9% acidic calcium sulfate, and either0.05% caprylic acid, 0.1% sodium benzoate, or 0.5% butyric acid killed > 5 log10 E. coli O157:H7, O26:H11, andO111:NM/mL within 30 min in water containing rumen content (Zhao et al., 2006). To avoid the substantial reductions by animals in their intake of treated water, it has been recommended that application of chemicals be periodic and that flushing be applied to remove or dilute the chemicals after 30 min of exposure. To maintain drinking water for chickens to be Campylobacter-free, on the other hand, acidification of drinking water with a commercial organic acid, prepared at concentrations recommended by the company,

is effective (Chaveerach, 2004). The digestive epithelial cells of chickens drinking the acidified water are not damaged and total consumption of the water is reduced. Consumption of 15mMchlorate-treated drinking water, however, does not affect consumption, yet significantly reduces the incidence of Salmonella in the crop and ceca of broilers (Byrd, 2003).

1.11 Farm Air (Bio-aerosols)

Air hygiene is an important factor to be considered in intensive poultry breeding as it has considerable impacts on the health of both animals and humans working in this industry. The air in animal facilities can be a reservoir of primary and potentially pathogenic microorganisms involved in the etiology of infectious and allergic diseases (Wathes, 1994). It is known that air in poultry houses is polluted with large quantities of different microbial components and metabolites, i.e. aggregation of bacterial and fungal cells, endotoxin (lipopolysaccharide, LPS) of Gram-negative bacteria, 1,3-beta-glucan of fungi, fungal spores and fragments of mycelium (Dutkiewicz,1987;Stetzenbach,1992). They are suspended as bioaerosols and can occur as liquid droplets or as dry particles. This bioaerosol may contain representatives of bacterial genera: *Pseudomonas, Bacillus, Corynebacterium, Pasteurella, Vibrio, Enterobacter, Salmonella, Brucella, Leptospira, Haemophilus, Mycoplasma, Yersinia, Staphyloccocus, Streptococcus, Micrococcus, Pantoea, and Sarcina*(Sieminski,2001;Vu

emilo, 2005). Bacteria in poultry dust bioaerosols may be derived from soil, feed, and bedding and from the birds themselves (fecal or skin microflora). Their presence in large numbers may represent a significant immunological challenge to the human respiratory system.

Many authors have assessed the content of air pollution on poultry farms and its effect on poultry health and productivity. (Vu

emilo,2006)found the concentration of airborne microorganisms in a poultry house to rise with poultry age, ranging from 3.22 × 103 cfu/m3 air in the first week to 6.40 × 107 cfu/m3 air in the fifth week of intensive breeding. The air level of ammonia was 14.8 ppmin the fifth week of fattening. Similar values of airborne microbiological contamination in the facilities of intensive poultry breeding were also reported in an earlier study (Vučemilo, 2005).One of the most important contaminants in the farming environment are bioaerosols. Animals and farm workers are exposed to high concentrations ofbacteria and fungi as well as endotoxins and mycotoxins produced by them (Barabaz, 2001; Nikulin, 1994). Airborne microorganismsmay cause various negative effects, especially infectious, allergenic and immunotoxin diseases. The concentration and kind of microorganisms in the indoor air depends on technical factors (i.e. type and age of a building), number of inhabitants (people or animals), the type of heating and ventilation systems, and microclimatic conditions: temperature, humidity, concentration of gases, lighting or dust concentration (Shaffer, 1997). Improper working methods and hygienic conditions may be causes of considerable microbial air pollution (Barabaz, 2001).

2.11.1 Rodents and rats

Prevention of food hazards in the first part of the food chain is essential to prevent illness of consumers. Control or better elimination of zoonotic pathogens (Jensen, 2004).In organic animal production systems, eliminations more difficult, as the animals are allowed outdoors and have easy access to potential sources of hazardous bacteria and/or parasites. Rodents that are present on organic farms can form one of these sources. Organic farms offer an ideal environment for them owing to the application of roughage and straw. Moreover, organic farmers are often

less willing to use rodenticides, since they perceive rodent presence as an integral part of the agro-ecosystem (Meerburg, 2006).

Whilst rodents are often associated with infrastructural damage and eating or spoiling of stored feed and products, their zoonotic risks are frequently underestimated. Wild rodents can be reservoirs and vectors of a number of agents that cause disease in food animals and humans (e.g. *Leptospira spp., Salmonella spp., Campylobacter spp., Trichinella spp., Toxoplasma spp*) (Gratz, 1994; Hiett, 2002).

Wild rodents are generally regarded as the main reservoir for Salmonella and Campylobacter in the environment. These warm-blooded animals can carry both bacteria in their intestinal tracts; mostly without showing any clinical symptoms of disease (Blaser, 1987). Infected animals can then cause transmission of pathogens from the farm environment to food animals, especially Campylobacter and Salmonella (Stern, 1992; Jacobs, 1994; Davies, 1995).Rodent's fecal pellets investigated for *Campylobacter* presence, revealed *campylobacter spp* (Fernie, 1977). The presence of rats on farms has been associated with an increased risk of Campylobacter introduction into broiler houses.15 Another study found that 87%of rat fecal samples tested were positive for *C. jejuni*.55Within poultry operations, Campylobacter can also be found in mice intestines(Hiett,2002).Author proved that, after artificial inoculation with *C. jejuni*. Rodents *M. richardsoni* can shed the bacterium for several weeks and have the potential to act as a reservoir in high mountainous areas (Pacha, 1977). No distinction has been revealed between organic and conventional systems. Someauthors31 found a high prevalent aminatence (24%) of *S. enteritidis* in commensal rodents present on cod chicken farms (Fernie, 1977).

Rodent control

Rodent control should therefore not only be applied by organic farmers to prevent economic losses but also from a veterinary perspective .Successful rodent management is built on three basic elements: preventive measures, monitoring and, if necessary, control measures (Meerburg,2004).Decontamination of farms is an important step in the reduction of pathogens (e.g. *Salmonella* and *campylobacter*) throughout the food chain. Although the disinfection procedure is the main risk factor for pathogen persistence after cleansing and disinfection, (Rose, 2000). The efficacy of a proper disinfection procedure is often reduced by the presence of Salmonella-infected mice remaining on or returning to the farm after cleansing, disinfection, and use of rodenticides periodically (Davies, 1995). Mice can acquire infections from inaccessible parts of the livestock houses or outdoor paddocks and then deposit contaminated droppings in places where food animals reside. Because of this food safety risk, even the smallest infestation with rats or mice on farms needs to be addressed (Davies, 1996; Meerburg, 2004).

Rodent control should form an integral part of a total package of hygiene measures. These should also include e.g. control of wild birds and flies and obligatory disinfection of boots,clothes and equipment for farm workers and visitors (Meerburg, 2007). Continuous monitoring of rodent populations and prevention (e.g. Proofing of farm buildings, removal of piles of old material, removal of habitat elements for rodents nears tables, limiting access to feed and water) will limit the development of high rodent densities on organic farms (Meerburg, 2007).

2.11.3 Pest, Flies and other insects

The carriage of bacteria by flies has been well established. Prevalence's of 50.7% and 43.2% were determined in a Norwegian study (Rosef and Kapperud, 1983) in flies sampled in the autumn from the

environs of poultry rearing units, respectively. Most isolates were *C. coli* (90.1%) with 6.2% being identified as *C. jejuni*. In contrast no surface sterilized insect sample was positive for the organism in another study (Jones, 1991). *Campylobacter* isolated from flies caught in broiler shed ante-rooms was of the same sero and biotype as the associated *Campylobacter* infected flocks (Berndtson, 1996).Flies exposed to infected chickens become contaminated within five days (Shane, 1985), and when these flies were transferred to a unit containing Campylobacter-free birds all birds became intestinally contaminated within eight days, and 70% had contaminated bile. In addition, water and litter became contaminated (Shane, 1985). Pest control, Presence of rodents or litter beetles has been correlated with increased campylobacter colonization of broiler(Berndtson, 1996; Kapperud, 1993; Refregier, 2001). Studies by Strother et al. (2005) illustrate the impact Pests can have as vectors of enteric pathogens.

1.11.4 Live bird handling and pre-slaughter stress

Handling of broilers is stressful operations that might affect welfare and meat quality and could increase numbers of deaths before slaughter (Quinn, 1998). Mechanical harvesting and unloading of containers had further improved welfare. Live birds handling causes stress, which leads to excessive faecal excretion (Berri, 2000). Recently a number of studies were performed providing information on the background of stress. Automated harvesting may not only reduce the physical damage to birds, but can also be a less stressful process (Quinn, 1998).

Pre-slaughter stressed animals have usually high temperatures, rapid glycolysis (pH drop), and early onset of rigor mortis in their muscles. Although the postmortem changes are rapid, some degree of ante-mortem muscle temperature rise, lactic acid buildup, and depletion

of ATP also occurs. This combination of conditions results in an exaggeration of the muscle-to-meat transformation (rapid pH decline and an elevated carcass temperature resulting in protein denaturation) that normally occur. Muscles from pre-slaughter stressed birds usually become pale, soft, and moist or exudative (PSE) after a normal 18 to 24 h chilling period condition. This condition most often results lower possessing yields, increased cooking losses, and reduced juiciness (Aberly et al., 2001).

Ante-mortem stress, including heat-stress (Babji, 1982), struggle before slaughter (Ngoka and Froning, 1982; Papinaho, 1995), have shown to accelerate glycogen depletion, increase the rate of pH decline, and possibly results in tough meat. Again, Glycogen deficiency usually occurs when animal survive stress, such that associated with fatigue, exercise, fasting, excitement, fighting or electrical shock but are slaughtered before they have sufficient time to replenish their muscle glycogen stores. Muscle glycogen deficiency in these birds' results in limited glycolysis in the muscles after death and results in a high ultimate ph. As a consequence of a high ultimate pH, changes in muscle color that otherwise occur during the post-mortem transformation of muscle to meat, do not occur. The pre-slaughter stresses normally occur in poultry meat processing industry are heat stress, pre-slaughter shackling, struggle, crating and transportation and feed withdrawal.

1.12 Pre-slaughter Heat stress

Heat stress is a major concern for poultry, especially in the hot regions of the world because of the resulting poor, growth performance, immunosuppression, and high mortality. Exposure to acute heat stress is likely to lead to various metabolic changes in poultry meat. An early reaction to high ambient temperature is the increased body temperature (Sandercock, 1999). Furthermore, according to Edens,1978), chicken

exposed to high environmental temperatures (43°C) showed a rising plasma corticosterone concentration early in the heating episode (before 90 min), afterwards a significant fall signifying the Acute Adrenal Cortical Insufficiency (AACI) syndrome. As reported by Edens and Siegel (1976) or Edens (1978), this syndrome is associated with a loss of plasma glucose, cholesterol, total calcium and inorganic phosphate and decreased plasma sodium to potassium ratio. Heat stress increases faeces production, so it is important that the animals can regulate their body temperature, and that no thermal imbalances are introduced (Brown, 1995).

1.12.1 Stress due to Struggling on the shackle line

Hanging operations could induce severe struggling(straightening up, wing flapping and vocalization) on the shackle line (Gregory and Bell, 1987), increase plasma corticosterone (Kannan et al., 1997; Debut et al., 2005) and affect muscle peri-mortem metabolism and some meat attributes (Debut et al., 2003). Struggling on the shackle line hastened the initial rate of the pH drop and increased the redness of breast meat struggling on the shackling line have been investigated in the context of animal welfare (Gregory and Bell, 1987; Gregory, 1994; Sparrey and Kettlewell, 1994). Kannan and Mench (1996) reported that hanging broilers in an inverted position is experienced as a stressful event which leads to an increase in plasma corticosterone concentration.

1.12.2 Feed withdrawal

Feed is normally withdrawn for several hours before catching in order to reduce the danger of carcass Contamination (Wabeck, 1972). Feed withdrawal varies according to eating patterns. Total feed withdrawal times of 8 to 10 h prior to slaughter are recommended (Wabeck, 1972). The emptying process of the crop occurs gradually in the 90- to 120-minute period after the bird has eaten (Shadbolt, 1994). Birds must have access to water during the withdrawal period: if the bird

does not drink, dry feed will remain in the crop indefinitely. Another potential problem is an increase in pH within the gastrointestinal tract during feed withdrawal, which promotes Salmonella growth. In addition, the intestinal strength starts to decrease after 12 to 14 hours off feed. The intestines become more easily broken after 18 hours off feed. Furthermore, in the absence of complex proteins, the caeca appear to become more friable and thus more susceptible to rupture during the evisceration process. To obtain full benefit from withdrawal, glycan may need to be added to the water used during the withdrawal period (Shadbolt, 1994).

1.12.3 Coops transport

Prior to processing, chickens are typically caught by hand, loaded into coops or crates, and transported on over-the-road trailers to the processing plant where they are held until slaughter (Lacy ; Czarick, 1998).The influence of transport, catching, and processing on contamination of broiler chickens with *Salmonella* and *Campylobacter* was investigated. Transport crates were reused with high frequency and were often still contaminated with Salmonella and Campylobacter when they arrived at the farm despite the fact that they were washed at the factory, and thus they were a potential route of infection (Slader, Bolton, 2001).During loading, transportation, and holding, cages become contaminated with feces, ingesta, dirt, feathers, litter, and other debris that may be carried into the processing plant on the birds' feet, feathers, and skin (Northcutt and Berrang, 2006). The processing plant must then work to remove these contaminants Crating, and loading are the procedures that are most likely to cause physical injuries, transportation has also been reported to be stressful to broilers (Duncan, 1989).In the little space available, birds tend to stand in an accumulation of their own

droppings. Cages with solid floors used during transportation enable birds to sit in accumulated droppings (Northcutt, 2006).

Although bacterial counts on poultry tend to decrease as carcasses progress through the processing plant, the initial microflora of live birds plays an important role in the microbiology of the final product (Jones,1991; Baker,1987;Izat,1988;Northcutt,2003).Unwashed or improperly cleaned transportation cages or crates can harbor pathogenic bacteria and then transfer these microorganisms to subsequent flocks .plastic transport coops before loading found that 98/99 (99%)were positive for *Salmonella* (Stern,1998).transportation in plastic crates resulted in a 2.5log10 increase in Campylobacter contamination of ceca. (Rigby, 1987; Stern, 1995; Altekruse, 1998; Jacobs, 1998; Berrang, 2003). Counts of bacteria (*Campylobacter, coliforms*, and *Escherichia coli*) on flooring were reduced by 1.5 to 2.0 log10 cfu/25 cm2after spray washing with tap water, and further reductions in counts did not occur when the flooring was immersed in a chemical treatment after the tap water washing (Northcut; Berrang, 2006).Coops were found to be frequently contaminated (57%) by *Campylobacter* prior to being used to transport birds (Hansson, 2005). Cleaning and disinfection, as practiced appeared to have little effect on the prevalence of contamination, with 69% of coops, modules and vehicles testing positive prior to cleaning and 57% positive after cleaning (McKenna , 2001).Transmission of *Salmonella* or *Campylobacter* may therefore happen from one flock to the next (Slader et al., 2002; Hanson et al., 2005). Transportation crates or containers may not be free of *Salmonella* after cleaning and disinfection (Corry, 2002) .The type of flooring of the crates (wire mesh or solid) had no effect on the presence of the pathogens on carcasses assessed after defeathering (Buhr, 2000).Drying of crates for 24 or 48 hours, with or without a spray wash, was effective in reducing the level

of Campylobacter contamination to an undetectable level (Berrang and Northcutt, 2006).

2.12.4 Poultry Transport (road)

Transportation and the micro-environments prevailing in coops and vehicles may impose varying degrees of stress upon the birds which willresult in compromise in of their welfare status, health and productive efficiencydepending upon the magnitude of the challenges impose (Mitchell 2006; Kettlewell , 2008) No effect of feedwithdrawal on the Campylobacter contamination of coops or broiler carcasses after short transportation periods (Jacobs,1999). (Gregory 1996) reviewed pre-slaughter handling systems, and referred to the studies of (Rigby; Pettit, 1980), who found an increasing number of birds shedding *Salmonella* during transport that lasted for a maximum of 4 hours. Longer transportation times lead to a lower prevalence. Reduction of stress was obtained by changing transport conditions: for instance the switch from small coops towards larger containers. Transport and holding prior to slaughter has been shown to increase both the prevalence and numbers of *Campylobacter* on chickens (Stern, 1995).Longer transportation times lead to a lower prevalence of fecal shedding, that reduction of stress was obtained by changing transport conditions (Brown, 1995).

1.13 Distortions in meat quality due to handling and transportation

Handling and transportation of live broilers to the abattoir were shown to be a major source of distortion of chicken carcass in the plant.

2.13.1 Physical injury

The incidence of physical injury may be determined after slaughter. The most frequent injuries seen are bruises, broken limbs and

damaged wings (Nicol, 1990). There is no doubt that catching, inversion, loading, transportation, unloading and shackling are painful, stressful and damaging processes (Elrom, 1999). The rate and types of injury depend on many factors. Manual handling has been identified as the most potential source of injury and stress to the birds (Kettlewell, 1994). It was mentioned by many investigators that more gentle catching crews had lower a score of physical injuries, (Frazer, 1990; Kettlewell, 1994). Moran, (1988) stated that the average incidence of injuries in the 1970's was 10-15% whereas Kettlewell and Turner (Kettlewel, 1985) estimated that the average was 5%, which is still a disturbing percentage

1.13.2 Bruising

Bruising provides an indication of the number and severity of physical insults sustained during transportation. Studies on broilers were concerned mainly with the economic consequences of bruising. Knowles and Broom, (1990) pointed out that according to several works, done in this field, the average incidence of bruising is between 2.63-20%. This wide range probably reflects the subjectivity of carcass grading and differences in inspection procedures. Damage of carcasses that may be caused during the processing must be differentiated from damage to the live bird. The incidence of bruising and crating has been correlated with average flock weight, daily temperature and sex (Knowles, 1990). Around the 70's it was shown that prolonged transportation of broilers led to a greater incidence of bruising than in broilers transported over shorter distances. Also the use of grater space systems increased the incidence of these injuries.(Gregory,1990) determined that bruises can be evaluated (aged) according to their color. Greening starts at 12-14 h after the injury, and so it should be possible to distinguish bruises from the growing period due to catching and handling.

1.13.3 Fractures and dislocations of bones

The pain associated with damage such as bone breakage is such that welfare could be considered as very poor while corticosteroids levels appeared to increase with increased number of brakes (Knowles, 1994). At points in the sequence of handling and transportation, the occurrence of bone breakage can be assessed by careful non-injuring killing (Knowles, 1990). Dislocated humerus bones are seen in broilers after transportation (. Gregory, 1994) and the numbers were estimated to reach 1.1% of 53,000 birds by Jespersen (Jespersen, 1996). Bremner and Jonston, (1996) pointed out that fractures in broilers were located mainly on femur, radius, ulna, furculum and ischium. They also add that the damage of catching plays only a minor part in the overall problem of broken bones (3%) and the processing may increase the percentage of fractures to 96%! Crushed skulls are found when plastic drawers in the modules are used and the catchers should therefore ensure that no heads are sticking out (Bremner, 1996).

By holding the bird with both legs the incidence of fractures when removing birds from cages is decreased. Finally, as mentioned before, differences in the incidences of bone fractures between loads are mainly due to better catching teams (Knowles, 1994).

1.14 Metabolic exhaustion and dehydration

The main outcome of transportation is metabolic exhaustion and dehydration, which are the result of interrelated, multifactorial stressful events and the bird’s response. Ante-mortem stress can have profound effects on meat quality mainly in the red meat species. The birds are metabolically exhausted after trying to cope with many events: wings flapping when loaded and muscle contraction due to

vibration, general metabolic stress reaction due to the complicated general stress state (thermal stress, psychological stress).

Dehydration is most commonly in sick, lame or undersized birds. These birds may not be able to reach the drinkers. Dehydration can be recognized from the skin being tough and difficult to tear by hand and the muscle has a sticky texture. In extreme cases there may be visceral gout (10).

Flocks with a history of congestive heart failure (CHF) are much more vulnerable if there is a progressive oxygen debt due to crowding, trampling or inadequate ventilation. CHF was found to be a major cause of mortality. In a survey it was found that CHF was accounted to be 47% of the deaths (of which 35% had ascites). In birds predisposed to CHF, acute heart failure may be the cause of death. CHF is recognized in D.O.A. birds by an engorged, massive heart and congestion of the lungs with blood (10).

1.14.1 Fecal shedding

Broilers are typically kept without feed for a few hours before loading. Feed deprivation is important in relation with the fecal shedding during transport and determines the amount of faeces present during slaughter. An optimum feed withdrawal time of 12 hours in relation to carcass contamination, without loss of bird weight (Zuidhof, 2004). Increasing fluid contents in the gut after feed withdrawal, may lead to more cross contamination especially in the case of slaughter failure (Warriss, 2004). The crop is increasingly considered an important source of contamination. During prolonged feed withdrawal periods, the Salmonella load in the crop increases. Broilers may start litter pecking, consuming feed remnants, faeces and small feathers. Moreover, there is a change in crop environment, whereby it becomes less acidic and more

attractive to pathogens like *Salmonella spp* and *Campylobacter spp* (Northcut, 2001).Liquid crop contents, although difficult to observe, have been reported to contribute to increased numbers of *E. coli* and *Campylobacter* on carcasses (Smith, 2005).Feed withdrawal affects the amount and the condition watery or firm of the digesta as well as the integrity of the gastro-intestinal tract. Watery gut contents can leak out more easily, posing a contamination risk during processing. In case of decreased gut integrity, there is a higher chance of gut damage and spillage of digesta during evisceration *Campylobacter* counts of freshly produced fecal material in transportation coops at arrival in the slaughter plant are higher than in fresh fecal samples in the broiler house before feed withdrawal (Nauta et al., 2007). This can be explained by the presence of relatively higher proportions of caecal material. significantly reduced Salmonella in the gut of broilers by administering a commercial chlorate product during the 10 h feed withdrawal period, although they did not monitor the effects during transportation or in the processing plant(Byrd 2003).the broilers may actually be more exposed to contamination as a consequence of excessive shedding of Salmonella during transported. Defecation during the early stage of the slaughter process appears to contribute in this respect, cross contamination in all processing steps (Hinton, 2004). Aerobic bacteria isolated from poultry feces were *E. coli*, *Pasteurellaspp*, *Bacillus spp*. and *Staphylococcus aureus*(Rahman, 2003). Effects of feed withdrawal are primarily negative from a contamination point of view (Debut, 2003).

1.14.2 Feather Contamination

Broilers feather contamination due to feaces present in the transport coop during loading (Berrang, 2000). Chicken feathers and feces are responsible for coop contamination instead of the coops contaminating the chicken feathers. *Campylobacter* become trapped in

folds and crevices of the skin particularly in the feather follicles (Corry and Atabay, 2001).Clean feathers reduce the bacterial load during the first processing steps; unloading, killing, scalding and plucking.

1.15 Cloacal isolates

1.15.1 Lairage time

Lairage time can be considered the interval between the arrival of the transport vehicles at the holding area and the unloading of the crates with birds into the unloading bay. To improve broiler welfare and reduce thermal stress after broiler transportation, holding areas with environmental control at the processing plants associated with a suitable lairage time interval can permit distribution of cooled air inside the crates before arrival at the slaughter line (Quinn, 1998).

Several authors have recommended short lairage times(less than 2 h) because of low energy availability infasted, metabolically active birds (Hunter, 1998;Warriss, 1999; Nijdam, 2004; Bianchi, 2005).

1.16 Bacteriology of Poultry Farm

1.16.1 Microbiology of Poultry

Contaminants may be micro-organisms that cause spoilage of the product or organisms of public health significance. Pathogens associated with poultry are *Salmonella, Staphylococcus aureus, Clostridium perfringens and Escherichia coli. Listeria monocytogenes and Campylobacter jejuni* have also been isolated from poultry. Spoilage bacteria most frequently associated with poultry are *Pseudomonas spp.,Acinetobacter, Moraxella, Alteromonasputrefaciens, Aeromonas spp., Corynebacterium, Flavobacterium, Micrococcaceae and*

Enterobacteriaceae. Poultry is a common vehicle of foodborne illness (Bryan, 1980; Todd, 1980;Smeltzer, 1981;Brown and Baird-Parker., 1982; Mead, 1982;Roberts, 1982; Ralph and Tompkin., 1984;Evans, 1986; Gill, 1986; Grau, 1986; Silliker and Galois., (1986); Cunningham (1987); Banwart, (1989); Mead, (1989); Zottola and Smith., (1990); Jones *et al.,* 1991).

1.16.2 Bacterial Genera involved in Poultry

According to Bensteed, (1965) Thornton, (1968) Riemann, (1969) Buchanan and Gibbons, (1974) Cowan, (1974) Hobbs and Christian, (1974) Carter, (1975) Lahellic*et al.,* (1975) Hubbert*et al*., (1975) Dugid*et al*., (1978) and Jay (1986) many gram positive and gram negative bacterial genera which were encountered in poultry :

1.16.2.1 The Genera of Gram Negative Bacilli

1.16.2.1.1. *Escherichia*

Escherichia is a genus of family *Enterobacteriaceae*, facultative Gram negative organism. It's described as a coliform of the intestinal tract of man and other animals from which it might be found in soil, water and many other places in nature. *E. coli* and *AeroactorAerogens* are known as Gram-negative, short rods, lactose fermenter. *E. coli* was reported as the most important entero-pathogenic coliform and differentiated from *A. aerogenes* by IMVIC reaction. This common lactose fermenting faecal genus shown to have serotypes pathogenic for humans. They are frequently reported in the literature and are known as entero-pathogenic *E. coli* (EPEC).

E. coli strains are a normal component of animal and human intestinal microflora, and thus serve as an indicator of fecal contamination in feed (Georgnaras et al., 2001). Whereas most cases of

human colibacillosis are mild or self-limiting, outbreaks of E. coli strain O157:H7 have gained recent public attention as it is a potentially lethal foodborne pathogen (Tauxe, 2002). Different strains of E. coli can cause a wide variety of disease syndromes in animals including septicemia, swollen head syndrome, cellulitis, and air sacculitis (Geornaras et al., 2001; Gomis et al., 2003; Miller et al., 2004). Some serotypes of E. coli (O2, O (21, 83), O78, and O115), have been linked to avian cellulitis (Peighambari et al., 1995; Elfadil et al; Norton, 1997).

1.16.2.1.1.1 Disease form in poultry

Named in poultry with colibacillosis, *Escherichia coli* infection or colisepticemia .Although most *E.cli* serotypes are nonpathogenic, alimented number produce extra intestinal infections ,pathogenic strains are commonly of the O1,O2,and O 78 serotypes , but serotypes O11,O15,O18,O51,O115 and O132have also been reported for *E.coli* isolates associated with cellulitis and colibacillosis.

Large numbers of *E.coli* maintained in poultry house environment through fecal contamination .initial exposure to *E.coli* may occur in the hatchery from infected or contaminated eggs, but system infection requires predisposing environmental factors or infectious causes .Mycoplasmosis ,Infectious bronchitis ,New castle disease , Hemorrhagic enteritis , precede colibacillosis. Poor air quality and other environmental stresses may also predispose to *E.coli* infection (The Merck veterinary manual, tenth edition, 2010).

Systemic infection occurs when large numbers of pathogenic *E.coli* gain access to the bloodstream from the respiratory tract or intestine. Bacteremia progresses to septicemia and death, or the infection extends to serosal surfaces, pericardium joints, and other organs (The Merck veterinary manual, tenth edition, 2010).

1.16.2.1.1.2 Clinical finding and lesions

Signs are nonspecific and vary with age , organs involved and concurrent disease .young birds dying of acute septicemia have few lesions except for an enlarged ,hyperemic liver and spleen with increased fluids in body cavities .Birds that survive septicemia develop sub-acute fibrinopurulent airsaculitis ,pericarditis ,perihepatitis ,and lymphocytic depletion of the bursa and thymys.Sporadic lesions include pneumonia ,arthritis ,osteomyelitis, peritonitis, and salpingitis(The Merck veterinary manual, tenth edition,2010).

1.16.2.1.1.3 E.coli infection in man

Strains are pathogenic and cause characteristic diarrheal symptom such as *E.coli* 0157:H7 which was isolated in 1975 in USA (Atiya, 2003).

The diseases caused by the pathogenic E-coli range from mild diarrhea to hemorrhagic colitis characterized by blood-stained diarrhea usually without fever but accompanied by abdominal pain. It is also a cause of the hemolytic uraemic syndrome, commonest in infants and young children, and characterized by acute renal failure and hemolytic anaemia (Anon, 2000).

Symptoms include abdominal pain, vomiting, anemia, thrombocytopenia, acute renal injury with bloody urine, seizure and pancreatitis (Atiya, 2003).

1.16.1.2 *Klebsiella*

Klebsiella is a genus of the family *Enterobacteriaceae*. This genus is Gram-negative rods, non-motile, capsulated, aerobic abdfacultatively anaerobic, catalase positive, oxidase negative and attacked sugars fermentative. This genus is among the infections due to miscellaneous micro-organisms. *Klebsiella* was the predominant flora in faecal samples from outbreak of poisoning involves 30 students (Riemann, 1969).

2.16.1.3*Proteus*

Proteus is a genus of the family Enterobacteriaceae. This species is found in the intestinal tract of man and animals. They are Gram-negative, motile, urease -positive. *P. vulgaris* and *P. morganii* produce hydrogen sulphide in abundant quantities, to liquefy gelatin and to swarm on moist agar. Outbreaks of food poisoning were ascribed to *Proteus*.

1.16.1.4 *Pseudomonas*

Pseudomonas is a genus of the family Pseudomonadaceae. Gram-negative rod. It can be recognized by its production of a blue green fluorescent pigment (pyocyanin), which in agar cultures, will diffuse into the medium. And they are aerobic. *P.aeruginosa*, however, is capable of growth at 41-420 C and the blue-green pigment (fluorescein) produced by other species of fluorescent pseudomonads found in water. It is also capable of growing anaerobically in stab cultures nitrate agar (WHO, 1997). The species is widely distributed in nature in soil and water, plants in the intestinal tract of man and other animal. These were found to be the most important bacteria in the low temperature spoilage of food such as meat and poultry. They were producing water-soluble pigment causing metallic sheet. An epidemic involving 409 cases of acute enteritis were reviewed *P. aeruginosa* was isolated from many patients (Riemann, 1969).

1.16.1.5 *Salmonella*

Salmonella is a Gram-negative rod-shaped, nonsporeforming organism. There is a widespread occurrence in animals, especially in poultry and swine, motile bacteria of the family *Enterobacteriaceae*. Non-motile exceptions are *S.gallinarum* and *S. pullorum*.which occur in the intestinal tract of humans and in warm-blooded and cold-blooded animals. To date, more than 2,300 serotypes of Salmonella are. Known to exist and new serotypes are being discovered each year. Of these

recognized serotypes, only about 100 are routinely isolated from food, animals and man (Guthrie, 1991). Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, animal feces; raw meats, raw poultry, and raw sea foods (Centers of Disease Control [CDC], 2002).Salmonella are the main cause of food poisoning from poultry meat (Dougherty, 1976; Todd, 1980). Little is known about the incidence of Salmonella in South Africa although figures have been reported by (Bok, 1986;Georgnaras, 1996). There are many sources from which poultry may obtain *Salmonella*, the main sources being from cross-contamination during breeding, hatching and intensive rearing operations. Salmonellas are not part of the normal intestinal microflora of poultry, but are acquired from the farm environment via insects, rodents and birds. Feed is also an important source of salmonellas through contamination of various components of the feed mix (Georgnaras, 1996).

1.16.1.5.1 Salmonella infection in poultry (disease form)

Salmonella infections are categorized as nonmotile serotypes (*S.pullorum, S.gallinarum*) and the many motile paratyphoid salmonella. Nonmotile salmonella and Salmonella paratyphoid infections in poultry are relatively common and have public health significance because of contaminated poultry products consumption (The merck veterinary manual, tenth edition).

The transmission can be vertical (transovarian) but also occurs via direct or indirect contact with infected birds or contaminated feed, water, or litter. Infection transmitted via egg or hatchery usually results in death during the first few days of life up to2-3 week of age. Transmission between farms is due to poor biosecurity (The merck veterinary manual, tenth edition).

1.16.1.5.2 Etiology of Salmonellosis

Pullorum disease caused by *Salmonella pullorum***,** fowl typhoid caused by *Salmonella Gallinarum*, and paratyphoid infection can be caused by any one of the many non-host adapted *Salmonellae*. These Salmonella infect many types of birds, mammals, reptiles, and insects. Paratyphoid infections are of public health significance via contamination and mishandling of poultry products. *Salmonella Typhoimurum, S.Enteritidis*, *S.Kentuckey,* and *S.Heielberg* are among the most common Salmonella infection in poultry. Some species or strains are more pathogenic than others. The prevalence of other species varies widely by geographic location and season (The Merck veterinary manual, tenth edition, 2010).

1.16.1.5.3 Common clinical findings and lesions

Birds may die in the hatchery shortly after hatching. Affected birds huddle near heat source, are anoretic, appear weak, and have whitish fecal pasting around the vent diarrhea. Survivors are small in size and become asymptomatic carriers with localized infection of ovary. Some of the eggs laid by such hens hatch and produce infected progeny (The Merck veterinary manual, tenth edition, 2010).

Lesions in young birds include unabsorbed yolk sacs and classic gray nodules in liver, spleen, lung, heart, gizzard, and intestines. Firm, cheesy material in ceca (cecal core) and raised plaques of the mucosa of lower intestine are sometimes seen. Occasionally, synovitis is prominent. Adult carriers usually have no gross lesions but may have nodular pericarditis, fibinous peritonitis, or hemorrhagic atrophic regressing ovarian follicles with caseous contents. In fowl typhoid addition lesions include a swollen, friable, and often bile-stained liver with or without necrotic foci, enlarged spleen and kidneys, anemia and enteritis. In paratyphoid, infection may localize in eye and synovial tissues.

Conversely lesions due to acute death caused by septicemia (The Merck veterinary manual, tenth edition, 2010).

The organisms occur more often in the caecum than in any other region of the gut from where they may be excreted for varying periods, without the host showing any sign of disease (Morris and Wells., 1970; Mead, 1982; Grau, 1986; Silliker and Galois., 1986; Mead, 1989; Zottola and Smith., 1990; Jones et al., 1991). Salmonellas from one flock can contaminate another, usually during conditions of intensive rearing and also when there is inadequate cleaning and disinfecting of the multi-cage transportation used to convey the birds to the abattoir. Studies have also shown that live poultry transported from the farm often introduce *Salmonella* into the processing plant. Such contamination may result in considerable scattering of *salmonellae* during processing especially in the plucking machines and the scalding tank and may lead to contamination of the final product (McBride et al., 1980; Mead, 1982; Mead, 1989;James et al., 1992).

1.16.1.5.4 Control of salmonellosis in poultry farming

General control measures for the *salmonella spp*, include strict sanitation in the hatcheries, fumigation of the hatching eggs, pelleting of feed, cleaning and disinfecting of poultry houses, rodent control, and use of competitive exclusion products. Maintenance of poultry in confinement and exclusion of all pets, wild birds and rodents help prevent introduction of infection. Several antibacterial agents help prevent mortality but cannot eliminate flock infection and may lead to drug resistance. The NPIP includes S.Enteritidis control measures in breeders, including depopulation of infected flocks, cleaning and disinfection of pullet and layers houses (The merck veterinary manual, tenth edition, 2010).

1.16.1.5.5 Salmonellosis in man

Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, animal feces, raw meats, raw poultry, and raw seafood's (Centers of Disease Control (CDC), 2002). *S. typhi* and the paratyphoid bacteria normally can cause septicemia or produce typhoid or typhoid-like fever in humans.

They also cause salmonellosis, a disease with milder symptoms (CDC, 2002). Salmonellosis is considered a fast infection. The organism grows and produces an endotoxin that cause the illness. Acute symptoms; Nausea, vomiting, abdominal cramps, minor diarrhea, fever, and headache. Chronic consequences; arthritic symptoms may follow 3-4 weeks after onset of acute symptoms. Incubation period: 6-48 hours (CDC, 2002). Infective dose; as few as 15-20 cells; depending upon age and health of host, and strain differences among the members of the genus. Duration of symptoms; Acute symptoms may last for 1 to 2 days or may be prolonged, again depending on host factors, ingested dose, and strain characteristics (CDC, 2002).

1.16.1.5.6 Salmonella: A public health perspective

Salmonellosis is an important global public health problem causing substantial morbidity, and thus also has a significant economic impact. Although most infections cause mild to moderate self-limited disease, serious infections leading to deaths do occur (Jong and Ekdahl, 2006). In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, foodborne diseases still dominate as the most important public health problem in most countries (Dominguez et al., 2002). Many foods, particularly those

of animal origin, have been identified as vehicles for transmission of these pathogens to human beings and spreading them to the processing and kitchen environment (Uyttendaele et al., 1998). In developed countries food is recognized as the most frequently implicated vehicle of transmission and causes heavy financial burden on health care systems (Jordan et al., 2006). In the United States alone, an estimated 1.4 million non-typhoidal Salmonella infections, resulting in 168 000 visits to physicians, 15 000 hospitalizations and 580 deaths occur annually and the total cost associated with Salmonella is estimated at US$ 3 billion annually (WHO, 2005). Apart from the foodborne infections, the other major epidemiological development in Salmonellosis is the emergence of multiple-antibiotic resistant Salmonella, particularly in the developing countries (Okeke et al., 2005).

1.16.1.6 *Shigella*

Shigella is a genus of the family Enterobacteriaceae. Shigella organisms are Gram-negative rod, non-motile, aerobic and facultatively anaerobic, catalase positive, oxidase negative, sugar fermenting without gas production and citrate negative. *Sh. Flexneri* and *Sh. Sonnei* are the common cause of dysentery in Britain and Musca domestica was reported to be the common mechanical vector transferring the organisms from faeces to food. The species of this genus occur in nature, polluted water and the intestinal canal of man where they cause bacillary dysentery. Shigellosis or bacillary dysentery was reported as human disease caused by members of the genus *Shigella* which included four serological distinct species *Sh. dysenteriae*, *Sh. Flexneri, Sh. Boydii and Sh. Sonnei*. The spread of the infection was shown to be by the faecal-oral route from person to person via the hands or contaminated objects.

1.16.1.6.1 Shigellosis in man

Symptoms: Abdominal pain; cramps; diarrhea; fever; vomiting; blood,pus, or mucus in stools; tenesmus. Onset time: 12 to 50 hours after ingestion of contaminated water (CDC, 2000). Infective dose: As few as 10 cells depending on age and condition ofhost. The Shigella spp. are highly infectious agents that are transmitted by the fecal-oral route (AWWA, 2000).

1.16.2 The Genera of the Gram Positive Cocci

1.16.2.1*Staphyloccus*

Staphylococcus is a genus of the family Micrococcaceae. Gram positive, nonmotile, facultative anaerobes, capable of producing enterotoxins in human and animal hosts (Bergey's Ninth Edition, 1994). *Staphylococcus spp*, have been routinely observed to be the highest percentage of the total bacterial population in litter. (Alexander ,1986) found *Staphylococcus* in 44 out of 44 litter samples(Lu,2003).It is also found in the nasal cavity and skin of man and certain other animals. The coagulase-positive members of the genus (e.g. *Staphylococcus aureus*).

1.16.2.1.1 Staphylococcosis in poultry farming

Staphylococcosis is the disease that can affect a wide range of avian species include poultry. The disease condition associated staphylococcosis vary with the site and route of inoculation and can involve the bones, joints, tendon sheathes, skin, sternal bursa, navel, and yolk sac.

1.16.2.1.2 Etiology

Staphylococcus aureus is the most common isolated recovered from clinical cases. Most pathogenic strains have been coagulase –

negative *Staphylococcus*, including *Staphylococcus hyicus*, *Staphylococcus epedermidis*, and *S.gallinarum* have been reported from clinical cases (The merck veterinary manual, tenth edition, 2010).

1.16.2.1.3 Transmission, epidemiology, and pathogenesis

Because Staphylococcus is part of normal skin and mucosal flora, many infections are result of a wound, mucosal damage, or both. Infections can also occur in the hatchery as a result of contamination of an open navel. Birds that are immunocompromised are also more prone to *Staphylococcus* infections. Once in the host S .aureus invades the metaphyseal area of the nearest joint, which leads to osteomyelitis and localization within that joint. Alternatively, the bacteria can invade the bloodstream and lead to a systemic infection in multiple organs (The Merck veterinary manual, tenth edition, 2010).

Food poisoning from poultry meat caused by *Staphylococcus aureus* is much less common than that due to *salmonellas* or *Clostridium perfringens* (Todd, 1980; Mead, 1982). *Staphylococcus* is important in relation to poultry meat, because it can produce enterotoxins which may cause food poisoning in humans (Notermans, 1982). Live poultry carry *Staphylococcus aureus* on skin surfaces and in nasal cavities, but low numbers are also present in the intestinal tract (Todd, 1980; Evans, 1986; Grau, 1986; Mead, 1989). Isolates of *Staphylococcus aureus* from poultry can be subdivided into human, non-human and intermediate types (Gibbs et al., 1978; Mead 1989). It appears that *Staphylococcus aureus* may also be obtained from human sources after hatching and during processing of the carcasses (Gibbs *et al*., 1978; Mead, 1982). Notermans*et al*., (1982) indicated that after processing, contamination of carcasses with this organism increased to >103 g-1 of skin. Defeathering machinery in particular may support the buildup of Staphylococcus aureus. Evisceration and chilling are also processing stages which have

been incriminated in contaminating carcasses with Staphylococcus aureus *(*Gibbs et al., 1978; Todd, 1980; Mead, 1982; Notermans et al., 1982; Mead, 1989).

2.16.2.2*Streptococcus*

Streptococcus is a genus in the family *Streptococaceae* ,nonmotile , gram positive , catalase negative coccoid bacteria that occur in pairs , or in short chains when observed on stained smear (merck veterinary manual,tenth edition,2010). Some species were reported to be associated with the upper respiratory tract of man and other animals causing scarlet fever and septic sore thorax. Others were in the intestinal tract of man and animals. Dutta and Devriese, (1982) reported *Streptococcus faecalis, Streptococcus faecalis subsp*. liquefaciens, *Streptococcus faecium*, and carboxyphilic streptococci in many poultry farms.

1.16.3 The genera of Gram Positive Bacilli

1.16.3.1 *Campylobacter jejuni*

Campylobacter jejuni is a Gram-negative cylinder, curved, spiral-shaped, and motile rod. It is a microaerophilic organism, which means it requires law levels of oxygen. It is relatively fragile and sensitive to environmental stresses (e.g. 21% oxygen, drying, heating, disinfectants, and acidic conditions). Because of its microaerophilic characteristics the organism requires 3 to 5% oxygen and 2 to 10% carbon dioxide for optimal growth conditions. This bacterium is now recognized as an important enteric pathogen (CDC, 2003).

Campylobacter in animals other than broilers in many animal species, Campylobacter spp. occur as commensals in the gastrointestinal tract. Campylobacter jejuni is predominantly found in poultry but has also been isolated from cattle, pigs and sheep (Manser&Dalziel, 1985; Quinn et al., 1994; Stanley et al., 1998; Stanley & Jones, 2003).

Campylobacter coli is predominant in pigs but has also been isolated from poultry, cattle and sheep (Manser&Dalziel, 1985; Nielsen et al., 1997). Thermophile *Campylobacter spp* have not been associated with gastroenteritis in production animals, but C. *jejuni*, *C. helveticus*, *C. lari* and *C. upsaliensis* have been isolated from both diarrhoeicand healthy dogs and cats (Stanley et al., 1992; Moreno et al., 1993; Hald&Madsen, 1997; Sandberg et al., 2002; Olsson Engvall et al., 2003). Campylobacter coli has not been described as a cause of disease either in production or pet animals

Poultry is a major reservoir of *Campylobacter jejuni*. Many commercial poultry flocks appear to be symptomless carriers of *C. jejuni*, with up to 107.g-1 of gut content being demonstrated in the ileum and caeca of infected poultry and similar levels in the feces (Genigeorgis et al., (1986) ; Mead, (1989) ; Zottola and Smith., (1990). Some poultry flocks that are negative before slaughter will therefore become contaminated during processing. Campylobacter is micro aerophilic with a relative high minimum growth temperature (30°C) and there seems little likelihood of them multiplying in the processing plant or on the raw, processed product.

The main problem in processing is that of cross-contamination (Zottola and Smith (1990); Smeltzer, (1981). Campylobacter species are more sensitive than many other organisms to the adverse effects of environmental conditions (drying, freezing and cold storage). For this reason, attention has been given to factors influencing the survival of *campylobacters* in processing.

Although freezing is harmful to Campylobacter, it does not eliminate this organism from poultry. Nevertheless, the contamination rate tends to be higher in fresh than in frozen carcasses. Campylobacter spp. are also more sensitive to chlorine than E. coli, but are not

eliminated from poultry carcasses by immersion chilling in chlorinated water. On the contrary, cooling-water seems to be an important reservoir of this organism: 100-3000 CFU.ml-1 were demonstrated and survival over long periods at low temperatures is possible. Campylobacter was also isolated from air samples as well as equipment (Cunningham, 1987; Mead, 1989; Zottola and Smith., 1990).

1.16.3.1.1Avian Campylobacter Infection

The organism colonizes the intestine of chickens, turkey, and waterfowl but nonpathogenic in mature poultry .some strains of *C.jejuni* have been reported to cause enteritis and death in newly hatched chicks and poults; however, it has not been possible to satisfaykoc's postulates and reproduce the syndrome previously termed "Avian vibrinoic hepatitis "by administrating isolate C.jejuni to chickens (The merck veterinary manual, tenth edition).

Commercial poultry and free living birds are natural reservoirs of the thermophelic campylobacters (*C.jejuni*, *C.coli*, and *C.lari*) and other poorly defined species .C.jejuni has been demonstrated in all areas of commercial poultry production. Campylobacter jejuni is the predominated species associated with foodborne infection derived from poultry .*C.lari* and *C.coli* can also be recovered from the intestinal tract of the poultry and have also been implicated in foodborne infection (The merck veterinary manual, tenth edition).

Environmental contamination is probably the most common source of infection for poults, chicks, and ducklings. Litter can remain infective for long periods, subject to at least 10% moisture level and neutral PH.

1.16.3.1.2Disease form and clinical findings

Many chicks recolonized with campylobacter spp early in life with no associated clinical signs or pathology .highly pathogenic isolates

derived from people with enterocolitis may induce some mortality in chicks.

Gross lesions in challenged chicks may include distinction of the jejunum disseminated hemorrhagic enteritis, and in some cases focal hepatic necrosis (The Merck veterinary manual, tenth edition, 2010).

1.16.3.1.4 Campylobacteriosis in man

The disease caused by *Campylobacter spp* (*jejuni* and coli). These bacteria are now recognized as an important enteric pathogen. Surveys have shown that C. jejuni is the leading cause of bacterial diarrheal illness. It causes more disease than *Shigella* species (spp) and *Salmonella spp*. combined (CDC, 2003).

Campylobacteriosis is the name of the illness caused by *C. jejuni*. It is also often known as *campylobacter* enteritis or gastroenteritis (CDC, 2003) C. *jejuni* infection causes diarrhea, which may be watery or sticky and can contain blood (usually occult) and fecal leukocytes (white cells). Other symptoms often present are fever, abdominal pain, nausea, and headache and muscle pain. The illness usually occurs 2-5 days after ingestion of the contaminated water. Illness generally lasts 7-10 days, but relapses are not uncommon (about 25% of cases) (CDC, 2000). The infective dose of C. jejuni is considered to be small. Human feeding studies suggest that about 400-500 bacteria may cause illness in some individuals, while in others, greater numbers are required (CDC, 2003). Diengaardt (2004) said in his study "Campylobacter spp.,

mainly C. coli and C. jejuni, are recognized as significant human bacterial pathogens, being responsible for increasing numbers of gastroenteritis cases worldwide. Several reports have indicated that environmental waters are potential reservoirs and transmitting vehicles for these bacteria.

1.16.3.1.2*Bacillus*

Bacillus is a genus of the family *Bacillaceae*. It is aerobic, spore-formers in air, dust, soil, water, and on utensils and various foods. Many are as important in the spoilage of many foods held above refrigerator temperature. *B. cereus and B. mesentericus* were species reported to be involved in food-poisoning. The characters of members of the genus are aerobic, Gram-positive endospore producer and on culture media long chains were produced.

1.16.3.1.3 *Corynebacterium*

*Corynebacterium***,** a genus which is the *Coryneform* group. Members of the genus are found in the intestinal tract of man and animal and had been isolated from spoiling foods of various types. The described characteristics of the genus were Gram-positive rod showing granules and cloud-shaped swelling, non-spore formers, mesophilic and psychrophilic, non-motile and non-capsulated bacilli. The species *C. dephtheriae* is milk-borne.

1.16.3.1.4 *Listeriamonocytogenes*

*Listeria monocytogenes*is garm- positive, non-spore forming, rod shaped bacteria that form long filament on stained smear. *Listeria monocytogenes* is widely distributed in nature and the environment. These organisms are isolated from soil, vegetation and faeces of humans and animals, with poultry often being contaminated. Studies also indicated that 57% (20 of 35 samples) and 33% (17 of 51 samples) of

market poultry, respectively, contained *L. monocytogenes. L. monocytogenes* can multiply at refrigeration temperatures. Data also suggests that *L. monocytogenes* is more heat resistant in meat than *Salmonella*. The necessity of proper hygiene procedures in handling, processing and packaging of poultry is therefore emphasized (Zottola and Smith., 1990).

1.16.3.1.4.1Listeriosis in poultry

Listeriosis is caused by the *Listeria monocytogenes,* which has a worldwide distribution. Although many species of birds susceptible to infection, clinical disease in birds is rare and generally occur as septicemia or localized encephalitis. Young birds are more susceptible to the effects of the disease. Adult birds often have an acute septicemia form, while young birds tend to develop the chronic form of the disease (The Merck veterinary manual, tenth edition, 2010).

Transmission is via ingestion contaminated nasal secretions, feces, and soil. Infection cans also occur via inhalation and wound contamination. Because infected birds often do not have any clinical signs, they can serve as a reservoir to perpetuate listeriosis in a flock. This disease often occur secondary to other diseases (The Merck veterinary manual, tenth edition, 2010).

1.16.3.1.4.2Listeria infection zoonotic risk

Conjunctivitis due to *L.monocytogenes* has been reported in individuals handling apparently healthy but infected chickens. Human infections have also resulted from the consumption of contaminated poultry or ready-to-eat poultry products.

1.16.3.1.4.3Breast blisters

In chickens and turkeys, a bursa lined with synovial membrane normally exists over the anterior projection of the keel bone. When this bursa becomes inflamed by trauma or infection, fluid or

exudates accumulates and appears as a fluid-filled blister 1-3 cm in diameter. Causes of trauma to this bursa include poor feathering, hard flooring, and leg weakness, which is associated with increased recumbence. Some broilers have pointed keels, which can lead to increased bursal trauma, but as the size of their breast muscle increases and trauma decreases, lesion may regress. Infectious causes of sterna bursitis include *Mycoplasma sunoviae, Staphylococcus,* and *Pasteurellaspp* (The Merck veterinary manual, tenth edition, 2010).

1.17 Sources of bacterial contamination of poultry meat:

The Identification and control of aerobic bacteria were reported by Lillard (1990) to increase safety and quality of broiler carcasses. He also claimed that bacterial contamination was reduced significantly by commercial procedures implementing hygienic measures. Control of *enteropathogenic* bacteria was indicated by Zivkovic, *et al*., (1989).

The most important genera of bacteria known to occur in foods were given by (Jay, 1986). They were 29 in numbers and included *Acetobacter, Acinetobacter, Aeromnas, Alcaligenes, Altramonus, Bacillus, Brochothrix, Campylobacter, Citrobacter, Clostridium, Corynebacterium, Lactobacillus, Leuconstoc, Micrococcus, Moraxella, Pediococcus, Ptoteus, Pseudomonas, Salmonella, Serratia, Shigella, Staphylococcus, Streptococcus, Vibrio and Yersinia*. Their primary sources presented were, soil, water, plants, and plant products, food utensils, intestinal tract of man and animals, food handlers feeds, animal hides, air and dust.

Abdella, (1993) investigated the aerobic bacteria of carcasses and main edible viscera of poultry slaughtered in the State of Khartoum. He isolated bacteria of the following 20 Genera: *Campylobacter, Listeria, Bacillus, Kurthia, Staphylococcus, streptococcus, Micrococcus, Aerococcus, Escherichia, Erwinia, Klebsilla, Pseudomonas, Salmonella,*

Shigella, Citrobacter, Proteus, Enterobacter, Edeardsiella and Morganella.

Most of bacteria found on poultry surfaces were found to consist of flora that were present prior to slaughtering and they were picked up during defeathering, pinning from workers hands and knives, from eviscerating or cooling due to cross contamination (Nickerson and Sinkey, 1974).The sources of contamination in poultry processing plant (Frazier and Westhoff, 1978) were two types: exogenous and endogenous. The exogenous contamination of the skin and the lining of the body cavities occurred during washing, plucking and evisceration. The authors also stated in their book that sanitation of the housing of the birds before killing had some influence on the numbers of microorganisms on the skin at dressing.

Microorganisms present in food were described by Banwart (1981). They included those acquired during handling and processing and those surviving a preservation treatment and storage. Water activity as a factor could play a role in spoilage. Jay (1986) pointed out that it might be assumed that all microorganisms existed in water might be existed in foods. These genera included: Acinetobacter, Bacillus, Citrobacter, Micrococcus, Proteus, Pseudomonas, Serratia and Treptomyces.

The hazard of air borne contamination of foods was indicated by Gregry (1961). He claimed that pathogenic organisms might spread in air by food handlers during sneezing or coughing and deposit on meat surfaces. Bryan (1978) and Jay (1986) considered food handlers to be important source of contamination. The microflora on their hands and outer garments generally reflect the environment and the habits of the individuals. This flora consisted of genera found on any object handled by the individuals in the addition to those from water, dust and soil. There were several genera of bacteria specially associated with the hand

and nasal cavities and mouth, the most important of which were Micrococcus and Staphylococcus. A related factor to contamination was the way of holding of feet and heads of the fowl on the slaughter line (Jay, 1986). Heavy contamination could be reduced (Robert, 1987) if equipment were adequately cleaned and sanitized at short intervals. Hudson and Mead (1989) reported in their study that poultry carcasses acquired *listeria* mainly via contaminated surfaces and equipments.In their microbiological survey, (Schuler and Badenhop (1972) found that the packing materials might also represent an important source of contamination. Avian slaughter processing plants bacterial cell per cm square. Jay (1986) mentioned that the environment of poultry slaughter-house contained several genera of bacteria more than in the soil, water or other places. Among these were *Bacteroides, Escherichia, Proteus, salmonella, Shigella, Staphylococcus and streptococcus*. Hoop and Ehrsam (1987) concluded from their epidemiological investigations that *Campylobacter* infected broiler flocks resulted from inadequate protection and that the slaughtering process was responsible for contamination of poultry. Abdel Salam (1986) investigated the prevalence and properties of *Clostridium perfringenes* in broilers in the Sudan. Kraft, *et. al* (1966) reported that the microbial flora of fresh poultry consisted largely of Pseudomonas and other closely related Gram-negative bacteria as well as *Corynebacterium*, yeasts and other organisms. *Psychrotrophic* bacteria were studied by Lahellic, *et. al*. (1975). They did their studies on 5920 strains isolated from chicken carcasses. They found that bacteria of the genus Pseudomonas constituted 30.5 percent, *Acinetobacter* 22.7 per cent, *Corynebacterium*12.7 per cent with yeast, *Enterobacteriaceae* and other in lower numbers.

1.18 Pre-slaughter handling and transportation:

For transportation to the processing plant, birds are usually caged in batches. However, stress caused by transport, crowding and exposure to weather conditions may lead to an increased frequency of defecation and discharge of ceacal contents (Grau, 1986; Mead, 1982; Parry, 1989). On the other hand, cages with perforated floors allow birds at higher levels to contaminate birds at lower levels (Mead, 1982; Grau, 1986; Mead, 1989). There is evidence that stress occurring during transportation can increase the proportion of birds which are intestinal carriers of Salmonella (Mead, 1982). It is therefore usual to starve birds before slaughter in order to minimize faecal contamination of carcasses during transportation and processing (Anand*et al.*, 1989; Mead, 1989). During unloading, it is inevitable that some birds will struggle and flap their wings as they are hung on the shackles, and this results in a considerable scattering of dust and micro-organisms. The only effective control in preventing the spread of airborne contaminants is the complete separation of this area from the rest of the processing plant (Mead, 1982; Mead, 1989).

Chapter Two
Materials and Methods

2.1 Sample Collection

The study was conducted for a period of three months, from June 2011, to September 2011 at a modern poultry farm in Khartoum State. A total of 144 samples were collected from two separate pens, namely: from litter, cloaca, feathers, transport coops, coop rinse water, and chicken breast supports in the slaughter line just before stunning. The chicken and sites were sampled by the swab technique.

2.1.1 Litter Samples

Three litter samples were collected randomly from 8 different chicken sites, for a total of 24 samples. Samples were collected in 2011 each flock will be considered a chicken batch. The average age of the flocks will be 32 to 34 days. Samples were collected from each batch when the chickens were 18 to 24 days old. Swab samples were taken from litter by moistening each swab(company) with 10 ml sterile buffered peptone water (BPW).Samples were taken by swabbing the litter back and forth in parallel movements towards to the water-supply pipes on the left and the right side of the poultry house.

2.1.2 Chicken transport coop samples

Swab samples were collected at the processing plant from the internal and external surface areas of two transport coops for each batch of chickens. Two swabs were collected from each coop for a total 30 swabs per batch. After sampling, the swabs will be placed in 50-mL sterile tubes containing 20 mL BPW.

2.1.3 Chicken transport coop rinse samples

Rinse water (50 mol) samples from the coops were collected directly from the rinse machine.

2.1.4 Feather samples

Before starting the slaughtering process, 10 coops were separated from each batch, and feather samples will be removed from one chicken in each coop, for a total of 10 chickens per sample (approximately 4 g of feathers). The feathers will be taken by hand from the neck and the breast

using sterile gloves. The feather samples will be placed in sterile, 100 mL BPW will add and the bags will be shaken for 30 seconds.

2.1.5 Cloacal samples

Before starting the slaughtering process, 10 coops were separated from each batch, and cloacal samples were obtained from one chicken in each coop, for a total of 10 chickens per sample. The cloacal samples were collected using the same swab for two chickens. The five swabs were placed in sterile flasks to which 20 mL BPW add. The flasks were shaken for 30 seconds and 2ml of the liquid was transported to sterile flasks containing 18 ml Bolton broth.

2.1.6 Breast support line

Two swabs were taken from a 100-cm of breast support track line. Was designed to calm the chicken after hanging surface area of the breast area support line before the stunning bath. The swabs were placed in tubes with 10 ml PBW. After shaking the sample for 15 seconds.

2.2 Isolation and Identification Procedures

The identification of isolates was carried out according to Barrow and Feltham (1993) and Holt *et al.* (1994).Isolates of test organisms were obtained from the nine CCPs from slaughterhouse, using prepared nutrient agar, nutrient broth, MacConkey agar (MCA) and Blood Agar. The plates were incubated at 37^{o}C for 24hrs. Well isolated colonies obtained from agar medium and different broth cultures of Gram-negative and Gram-positive bacteria were constantly sub cultured intoagar slants from time to time, incubated at 37^{0}C for 24 hrs and stored at 40C. Identification was based mainly on the followings;

i/ Indole production (ii) Presence of catalase (iii) Haemolysis on blood agar (iv) Acid and gas production (v) Microscopic and macroscopic examination of morphology (vi) Gram stain. The methods TVC and

Identification of the different strains that used were as described by Harrigan and MacCance, (1966) Barrow and Feltham, (1993).

Table (1): Distribution of the 144 Samples Collected from Broilers Carcasses on the Processing Line

No.	Critical control point	Number of Samples
1-	Litter	24
2-	Chicken transport coops	16
3-	Coop rinse water	10
4-	Feather	10
5-	Cloacal samples	20
6-	Breast support line	20

3.2.1MacConkeys Agar

MacConkeys agar of (Oxoid*) contained peptone 20 grams, lactose ten grams, bile salts five grams, neutral red 0.075 grams and agar 12 grams. pH was adjusted to 7.4 approximately. Forty grams of the dehydrated medium were suspended in one liter of distilled water dissolved by boiling, then sterilized by autoclaving at 121 ^{0}C for 15 minutes under pressure 15 lb per square inch. The medium was dispensed in sterile petri dishes in * Oxoid Laboratory Products, London.

2.2.2 Liquid Cultural Media

a. Peptone water was prepared according to Cruikshnk*et al.* (1975). Ten gram peptone and five grams NaCl were dissolved by heating in 1000 ml distilled water. The pH was adjusted to 7.2 and the medium was distributed in five amounts in the test tubes and sterilized by autoclaving at 1150 C for 15 minutes under pressure 15lb per square inch. The stock was preserved in the refrigerator.

b. Nutrient broth (Oxoid*) contained lab-lemco powder one gram yeast extract two grams peptone five grams and sodium chloride five gram. The pH was adjusted to 7.4 approximately. An amount of 13 grams of the dehydrated medium was added to one liter of distilled water. The reconstituted medium was mixed well and distributed in five ml amounts and sterilized by autoclaving at 121^0C for 15 minutes under pressure 15 lb per inch.

2.2.3Solid Cultural Media:

2.2.4Nutrient Agar

2.2.5 Mannitol Salt Agar

Mannitol salt agar (Oxoid*) contained: lab-lemco powder one gram, peptone ten grams, mannitol ten grams, sodium chloride 75 grams, phenol red 0.025 grams, and agar 15 grams. An amount of 111 grams of the dehydrated medium was suspended in one liter of distilled water. The mixture was boiled to dissolve completely. Then sterilized by autoclaving at 121 ^{0}C for 15 minutes under pressure 15 lb per square inch, and distributed in sterile Petri dished 15 ml each.

Nutrient agar was obtained in a dehydrated form (Oxoid*) the medium contained, Lab-lemco powder one gram, yeast extract two grams, peptone five grams, NaCl five grams, and agar 15 grams per 1000 ml. pH was adjusted to 7.4 approximately. The medium was prepared by adding 28 grams of dehydrated medium to 1000 ml distilled water and dissolved by boiling and distributed in final containers and sterilized by

autoclaving at 115 ^{0}C for 15 minutes under pressure 15 lb per square inch.

2.2.6 Blood Agar

Blood agar was prepared according to (Barrow and feltham, 1993). Ten ml sterile defibrinated sheep blood was added to 90 ml nutrient agar which was melted and cooled to 50 ^{0}C. The blood agar after mixed well was distributed (15-20 ml) under flame into sterile petridishes and allowed to solidify at room temperature. The prepared plates were kept in the refrigerator.

2.3 Methodology of Viable Bacterial Cell Count

Total Viable Count (TVC) was carried out as described by Harrigan and Mac Cance (1976) and Göksoy*et al.* (2004).Serial dilutions were used, plating and counting of live bacteria to determine the number of bacteria in a given population was used. Serial dilutions of a solution containing an unknown number of bacteria were made. The total number of bacteria in the original solution was determined by counting the number of colony forming units and comparing them to the dilution factor. Each colony forming unit represented a bacterium that was present in the diluted sample. The numbers of colony forming units (CFU's) are divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria per mL that were present in the original solution.

2.4 Lab 2. Counting colony forming units and calculating the amount of bacteria in the original solution

For each dilution, the number of colony forming units on the plates was counted. Typically numbers between 30 and 300 are considered to be in the range where one's data is statistically accurate. Alternatively, if the numbers are evenly distributed on the surface of the plate. If the

number of CFUs on the plate is below 10, the number of CFUs has to be recorded but was not used in the calculations.

2.5 Statistical Analysis

The data were analyzed with SPSS software (Statistical Package for the Social Sciences, version 11.5, SSPS Inc, and Chicago, IL, USA). All bacterial counts were converted to $\log_{10}$ CFU/cm^{-2} for analysis and ANOVA was performed. Statistical significance was set at a P value of <0.05.

Chapter Three

3.Results

The study revealed a statistically significant difference at *P-value* ($p \leq 0.05$) between the investigated Critical Control Points CCPs, as shown in Table 1, the TVC revealed the highest contamination level recorded was in cloacal swabs $9.98 \pm 0.01 \log_{10} CFU/cm^2$ while the lowest contamination level recorded was in coops swabs $2.76 \pm 0.11 \log_{10} CFU/cm^2$.

Table (2): The total viable count of bacteria (Mean ± SD) in difference points.

Operational points	Mean ± SD of TVCs of bacteria ($\log_{10}$ CFU cm^{-2})	**Significance**
Litter sample	7.09±0.12	*
Cloacal sample	9.98±0.01	*
Feather sample	9.85±0.15	*
Coops	2.76±0.11	*
Coops rinse	6.14±3.05	*
Breast support	9.33±0.24	*

Isolation and identification of bacteria at different operational points under investigation revealed 4 species of bacteria as shown in Table 2. Litter was *Staphylococcus aureus* and *Staphylococcus albus*(8.33%), cloaca was *Escherichia coli* (8.33%)and*Salmonella* (8.33%), feathers *Escherichia coli* (8.33%) and*Staphylococcus aureus* (8.33%), transport coops *Escherichia coli* (16.66%), coop rinse water

Staphylococcus aureus (16.66%), and chicken breast supports *Escherichia coli* (8.33%)and*Staphylococcus albus*(8.33%).

Table (3): Bacteria species isolated in Different Operational Points

Total	***Salmonella***	***S taph. albus***	***St aph. Aureus***	***E. coli***	**Operational points**
2 (16.66%)	0 (0.0%)	1 (8.33%)	1 (8.33%)	0 (0.0%)	**Litter sample**
2 (16.66%)	1 (8.33%)	0 (0.0%)	0 (0.0%)	1 (8.33%)	**Cloacal sample**
2 (16.66%)	0 (0.0%)	0 (0.0%)	1 (8.33%)	1 (8.33%)	**Feather sample**
2 (16.66%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (16.66%)	**Coops**
2 (16.66%)	0 (0.0%)	0 (0.0%)	2 (0.0%)	0 (0.0%)	**Coops rinse**
2 (16.66%)	0 (0.0%)	1 (8.33%)	0 (0.0%)	1 (8.33%)	**Breast support**
12 (99.96%)	**1** (8.33%)	**2** (16.66%)	**4** (33.33%)	**5**(41.66%)	**Total**

Chapter Four
4.Discussion

The environment of broilers houses in modern poultry production system is usually contaminated with a huge number of different microbial components i.e. aggregates of bacterial and fungal cells and their spores, endo-toxins (lipopolysaccharide, LPS) of Gram-negative bacteria, 1,3-beta-glucan of fungi and fragments of mycelium (Dutkiewicz,1987;Stetzenbach,1992). Therefore, hygiene is an important factor to be considered in intensive poultry breeding, as it has considerable impacts on the health of both animals and humans working in this industry. Bacterial contamination on processed broiler carcasses originates from many sources including live broilers, plant equipment and environment, and plant employees (Izat*et al*., 1988; De Boer and Hahne, 1990; Jones *et al*., 1991). Contamination from the live animal may occasionally originate from internal tissues. The two important major sources are the gastrointestinal tract (internal) and the feathers and skin (external). Variations exist in the internal-external profile of different types of bacteria on broilers. Having more information at hand about the relative contributions of internal and external sources would be useful to develop new processing procedures to reduce total contamination on processed carcasses. Moreover, the environmental concentration of microorganisms in poultry housing reported in previous studies varied greatly (Vučemilo*et al.*, 2007). In the Sudan there are no standard regulations concerning the acceptable number of bacteria and fungi in broilers houses.

Previous studies have shown that poultry litter harbors abundant numbers of different pathogenic and non-pathogenic bacteria. The acceptable total litter bacteria concentrations fall within the range of 10^{10} to 10^{11}cfu/g of litter as has been indicated by Terzich*et al*. (2000). While the total aerobic bacteria counts were set at lower standard, from 10^{8} to 10^{10}cfu/g of litter, as has been indicated by Macklin *et al*. (2005) and Lu *et al*. (2003). In this study the mean TVCs obtained from the litter, 7.09±0.12$\log_{10}$cfu, was lower than that of Terzich*et al*. (2000), Macklin *et al*. (2005) and Lu *et al*. (2003). This dissimilarity could be explained by the differences of disinfecting protocols practiced in the studied establishments. Broilers' litteris a mixture of substrates with the feces of birds where many undesirable bacteria may develop, such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Clostridium perfringens*, and *Staphylococcus spp.* (Lu *et al.,* 2003; Bang *et al*., 2002; Dhanarani*et al*., 2009; Graham *et al*., 2009; Terzich*et al*., 2000; Vizzier-Thaxton*et al*., 2003). In the present study *Staphylococcusaureus* and *Staphylococcusalbus* were isolated from the litter. These findings are consistent with the reports of Lu *et al.* (2003) Bang *et al*. (2002), Dhanarani*et al*. (2009), Graham *et al*. (2009), Terzich*et al*. (2000) and Vizzier-Thaxton*et al*. (2003).

Numerous pathogenic bacteria species have been isolated from the cloacae of domestic and wild birds (Lombard *et al.,* 1998). After hatching, the intestinal tract of poultry becomes colonized by *Enterobacteriaceae*(including coliforms), *Clostridia*spp. and fecal *Streptococci* (Grau, 1986). Later, these organisms are replaced by a characteristic microflora in different regions of the intestines. *C. jejuni* can be present in the lower intestinal tract in populations as high as 10^{7} per g. It colonizes primarily the lower gastrointestinal tract of chicks, principally the ceca, large intestine and cloaca where densely packed

cells localized in mucus within crypts (Beery et al., 1988). Colonization levels in the small intestinal, especially the ceca, range from 10^5 to > 10^9cfu/g (Berndtson et al., 1992; Mead et al., 1995).In the present study *Escherichia coli* (8.33%) and *Salmonella* species (8.33%) were isolated from cloacal swabs confirming the reports of Grau(1986) and Lombard *et al.* (1998). While the mean TVC was 9.98±0.01cfu. The count of viable bacteria reported in this study is consistent with the findings of Berndtson *et al.* (1992) and Mead *et al.* (1995).

During loading and transportation of broilers from production sites to slaughterhouses, cages become contaminated with feces, ingesta, dirt, feathers, litter, and other debris which are considered major sources of bacteria like *Campylobacter*, coliforms, *Escherichia coli* and other bacteria. Unwashed or improperly cleaned transportation cages or crates can harbor pathogenic bacteria and then transfer these microorganisms to processed poultry meat and/or subsequent flocks (Northcutt and Berrang, 2006).Plastic transport coops before loading found that 98/99 (99%)were positive for *Salmonella*(Stern, 1998). In this study *Escherichia coli* (16.66%) and *Staphylococcus aureus* (16.66%) were isolated from transport coops and coop rinse water, respectively. These findings typify the findings of Northcutt and Berran, (2006). Interestingly, results of this study did not confirm the report of Stern (1998). Although bacterial counts on poultry tend to decrease as carcasses progress through the processing plant, the initial microflora of live birds plays an important role in the microbiology of the final product (Jones,1991; Baker,1987;Izat,1988;Northcutt,2003).Transportation in plastic crates resulted in a 2.5$\log_{10}$ increase in *Campylobacter* contamination of ceca (Rigby, 1987; Stern, 1995; Altekruse, 1998; Jacobs, 1998; Berrang, 2003). Counts of bacteria on flooring were reduced by 1.5 to 2.0 $\log_{10}$cfu/25 cm^2after spray washing with tap water and further reductions

in counts did not occur when the flooring was immersed in a chemical treatment after the tap water washing (Northcut and Berran, 2006). The means of TVC reported in this study from transport coops and coop rinse water were 2.76±0.11 and 6.14±3.05log_{10}cfu/25 cm^2, consecutively. The counts of bacteria on coops were clearly reduced after wash. These findings are in agreement with the findings of Rigby (1987), Stern (1995),Altekruse(1998), Jacobs (1998) and Berrang(2003).

Broilers' feather contamination from fecal materials occurs in the transport coop during loading and transportation of broilers from production sites to slaughterhouses (Berrang, 2000). The feathers and feet of broilers routinely contact feces on the litter in the grow out house. During transport and holding prior to processing, the breast feathers and feet are typically coated with freshly excreted feces. Rigby and Pettit (1980) stated that contaminated feces carried on feet and feathers are important routes for introduction of pathogen contamination into the processing plant. Clean feathers reduce the bacterial load during the first processing steps; unloading, killing, scalding and plucking(Corry and Atabay, 2001).Morar,(2008) reported that broilers feathers contributions to carcasses contamination were as follows: 5 log cfu/cm^2 total viable counts; 2 log cfu/cm^2 enterococci; 2 log cfu/cm^2sulphite reducing-anaerobes bacteria. In this study the mean TVC obtained was 9.85±0.15log_{10}cfu/cm^2which is very much higher than that reported by Morar(2008). *E. coli* and *Staph. Aureus* were isolated from feathers in the present study and this result is in agreement with Morar (2008) who isolated coliforms from broilers feather.

From breast support a mean TVC of 9.33±0.24log_{10}cfu/$cm^{-2}$with *Escherichia coli* (8.33%) and *Staphylococcus albus* (8.33%) being the isolated contaminant bacteria. To the best knowledge of the authors this

the first time breast supports to be investigated as a potential source of microbial contamination in broilers.

Conclusions

In conclusion, the levels of microbial contamination in broiler chicken farms may reflect the hygienic status of poultry meat production. Bacterial contamination onprocessed broiler carcasses may originate from environment, plant equipments and employees

Recommendations

Hygiene is an important factor to be considered in intensive poultry farms as it has considerable impacts on the health of both animals and humans working in the industry.

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