Sudan University of Science and Technology

College of Graduate Studies

Sources of bacterial Contamination in Parent poultry Houses in Khartoum State

مصادر التلوث في حظائر امهات الدواجن في ولايه الخرطوم

By

Mohamed Abdalla Mohamed

B.V.M. 2010

A Thesis Submitted in the Fulfillment of the Requirement of Master in preventive Veterinary Medicine (Public Health)

Supervisor

Prof.Dr. Mohamed Abdelsalam Abdalla

Department of Preventive Medicine and Public Health, College of Veterinary Medicine, Sudan University of Science and Technology

March 2015

بسم الله الرحمن الرحيم

(يرفعِ اللهُ الَّذين آمنوا منكم والَّذين أوتوا العِلمَ درجاتٍ)

صدق الله العظيم

(المجادلة آية 11)

Dedication

To my father ,

my mother,

my brothers and

sisters,

my fiancee,

my colleagues and

friends and

all those were in touch during work .

Alshaar

ACKNOWLEDGMENTS

Above all, praise is to my almighty Allah for giving me a good health, wisdom, ability, and strength to carry out this work and for all other graces.

I would like to express my deep and sincere gratitude and appreciation to my supervisor Prof. Dr. Mohamed Abdelsallam Abdalla, dean of the College of Veterinary Medicine, University of Science and Technology for his excellent guidance, support, and constant encouragement throughout this project and also for his invaluable assistance and instructions, without which, it would not have been possible to accomplish this project and for reading and correcting the manuscript.

I am also indebted to Dr. Siham Elias Suliman and Dr. Yassir Adam Shuaib, College of Veterinary Medicine, University of Science and Technology for their kind assistance and guidance and would like to thank them very much.

I would like to express my sincerest appreciation and deepest thank to the staff of the Department of Pathology, Microbiology and Parasitology and to the staff of Microbiology Laboratory for their significant contribution and providing scholarship, without which it would not have been possible to accomplish this project.

Finally, I am thankful to my beloved mother, father, sister and brothers, and to all my friends for their everlasting support.

Table of Contents

List of Tables

List of Abbreviations

Abbreviation Word

Total viable count	TVC
Hazard Analysis Critical Control Point	HACCP
World Organization for Animal Health	OIE
Food Safety and Inspection Service	FSIS
Centers for Disease Control and Prevention	CDC
National Aeronautics and Space Administration	NASA
Food and Drug Administration	FDA
Critical Control Points	CCPs
Immunomagnetic separation	IMS
Electrochemilu- minescence	ECL
Tripropyl amine radical	TPAz
Short chain fatty acids	SCFA
Xylose –Lysine Deoxycholate Agar	XLD-Agar
Stander Deviation	SD

Abstract

This work was conducted to identify the main points of contamination of parent (chickens) houses in Khartoum state . A total of 108 samples were collected randomly from the parent houses at age 25 , 35 and 45 weeks as followed 36 sample in 25weeks ,36 sample in35weeks and 36 sample in 45weeks from feeds, water, feathers, nests, litters and coloaca aseptically. The mean (TVCs) and isolation and identification of contamination bacteria were conducted.

The TVCs was high in the sample taken from the coloaca at age 35 and 45weeks (6.25 ±0.08, 6.28±0.06). The contaminated bacteria at different point of contaminated were *E. coli* and *Salmonella spp*. In conclusion, the source of contamination can be started from rearing of chick in parent houses, but the reduction of contamination associated sanitary measures.

ملخص

تم اجرى هذا العمل لتحديد النقاط الرئيسية لتلوث حظائر امهات الدواجن في ولاية الخرطوم . تم جمع 108 عينه عشوائية من احظائر امهات الدواجن في عمر 25 و 35 و 45 أسبوعا على النحو التالي 36 عينة من الاسبوع 25 ، 36 عينة في الاسبوع 35 ,36 عينة في الاسبوع 45 من الأعلاف والمياه و الريش، و الأعشاش ، الفضلات و مؤخرة الفراخ في جو معقم و مطهر. ومن ثم تم عزل والتعرف علي البكتريا الملوثه .

كان التلوث في الاعشاش اكثر تلوث في الاسبوع 25 (70.0 ±6.25) وكانت المؤخره اكثر تلوث في الاسبوع 35و 45 علي التوالي(0.08 ± 6.25 ,0.06 ± 6.28). وكانت البكتريا الملوثة في نقطة مختلفة هي السالمونيلا و اي كولاي.

وفي الختام , يمكن ان يبدا مصدر التلوث من حضانة الكتاكيت ,ولكن للحد من التلوث نعمل التدابير الصحيه الجيده.

Chapter One

Introduction

Food safety was identified as a high priority of area in the 2001-2005 by world organization for animal health (OIE) Strategic Plan. Member of countries in OIE considered that the organization should be more active in issues of public health and consumer protection and that should include more involvement in the area of diseases or pathogens transmissible through food. Food safety is not only an industry responsibility , but also a major consumer concern. As result of societies 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 . Meat and meat products of particular importance regarding food borne illnesses . Food borne pathogens can be introduced to foods during rearing, processing, storage and preparation, from infected humans who handle the food or by cross contamination from some other raw agricultural products. Epidemiological data suggest of that contaminated products of animal origin, especially poultry, contribute significantly to food borne diseases. Reduction of raw poultry contamination levels would thus have a large impact on reducing the incidence of illness. Each year, millions of people worldwide suffer from food-borne diseases and illness resulting from the consumption of

contaminated food, this has become one of the most widespread public health problems in contemporary society. Some microorganisms such as *Salmonella* spp and *Escherichia coli* pose a threat to consumer health. Food-borne illnesses in human beings due to bacterial pathogens and their toxins are well documented worldwide (Hazariwala *et al*, 2002).

Salmonella spp has been reported by the United States Department of Agriculture Food Safety and Inspection Service (FSIS) as one of the most common causes of food-borne illness associated with meat and poultry products (Rindhe *et al.*, 2008). Risk factors for flock colonization by *Salmonella* include season, hatchery of origin, feed mills and various hygienic measures.

E. coli has been the subject of intense research for over a century. Nowadays it is by far the best-studied bacterium and most extensively used model organism and laboratory workhouse in molecular biology (Gordon and Cowling, 2003). *E. coli* is also widely distributed in the environment and capable of surviving in soil, water and sediment (Savageau, 1983 , Power *et al* 2005, Ishii *et al* 2006, Walk *et al*, 2007). In humans, the majority of *E. coli* isolates are commensals of the lower intestinal tract (Tenaillon *et al.*, 2010), but *E. coli* also includes life-threatening, pathogenic strains that are responsible for diseases in the gut, urinary tract, blood stream, and central nervous system (Kaper *et al.*, 2004) . *E. coli* is an important emerging human pathogen causing Haemmorhagic Colitis, Haemolytic Uraemic Syndrome and Thrombotic

Thrombocytopaenic Purpura . at this bacteria are identified as enterohaemorrhagic *E. coli* . In 1982, an investigation by the Centres for Disease Control and Prevention (CDC) and two cases shoring severe bloody diarrhea associated with a fast food restaurant led to identification of unique *E.coli* strain . The infections by *E. coli* have been reported of increasing frequency from all parts of the world in the form of food poisoning outbreaks

Escherichia coli strain produces shiga toxin 1 (stx-1) and shiga toxin 2 (stx-2) which are also referred as verotoxins. *Escherichia coli* strains carrying stx genes along with enterohaemo- lysin (hlyA) and intimin (eae) genes are potentially dangerous to human health (Kiranmayi and Krishnaiah, 2010).Hazard Analysis Critical Control Point is a well accepted systematic program for identification and processing applied to the poultry industry to improve microbiological quality of broiler carcasses and reduce microbiological hazards from to consumption (Jensen ,1996).

The aim of this investigation to identify the sources of salmonella and E .coli in poultry farm by analyzing feeds, water, feathers, nests, litters and coloaca and to determine bacterial number (Total Viable Counts) on parent poultry houses .

Chapter Two

Literature Review

2.1 History of HACCP

HACCP has become synonymous with food safety. It is a worldwide-recognized systematic and preventive approach that addresses biological, chemical and physical hazard through anticipation and prevention, rather than through end-product inspection and testing.

The HACCP system for managing food safety concerns grew from two major developments. The first breakthrough was associated with W.E. Deming, whose theories of quality management are widely regarded as a major factor in turning around the quality of Japanese products (TQM) systems which emphasized a total system approach to manufacturing that could improve quality while lowering costs.

The second major breakthrough was the development of the HACCP concept itself. The HACCP concept was pioneered in the 1960s by the Pillsbury Company, the United State Army and the United State National Aeronautics and Space Administration (NASA) as a collaborative development for the production of safe foods for the United State space programmed. NASA wanted a zero defects programme to guarantee the safety of foods that astronauts would consume in space. Pillsbury therefore introduced and adopted HACCP as the system that could provide the greatest safety while reducing

dependence on end-product inspection and testing. HACCP emphasized control of the process as far upstream in the processing system as possible by utilizing operator control and/or continuous monitoring and techniques at critical control points. Pillsbury presented the HACCP concept publicly at conference for food protection in 1971.The use of HACCP principle in the promulgation of regulations for low canned food was completed in1974 by the United State Food and Drug Administration (FDA). In the early 1980s, the HACCP approach was adopted by other major food companies.

2.1.1 Principles of HACCP

For the purposes of this study, the following seven principles that are the basis of the HACCP system have been sourced from the Codex Alimen tarius Commission . Report of the 29th Session of the Codex Committee on Food Hygiene (1996) (Alinorm 97/13A) "Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for its Application".

2.1.1.1 Principle 1

Conduct a hazard analysis.

2.1.1.2 Principle 2

Determine the Critical Control Points (CCPs).

2.1.1.3 Principle 3

Establish critical limits.

2.1.1.4 Principle 4

Establish a system to monitor control of the CCP.

2.1.1.5 Principle 5

Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

2.1.1.6 Principle 6

Establish procedures for verification to confirm that the HACCP system is working effectively.

2.1.1.7 Principle 7

Establish documentation concerning all procedures and records appropriate to these principles and their application. MAF Regulatory Authority (Meat and Seafood) HACCP Steering Group Amendment 9: (August 2004).

2- **2 History of poultry In Africa :**

Domestic chickens are closely associated with humans, and they rely entirely upon humans for their dispersal and indirectly for their survival. The species are therefore important biological markers of agricultural, trade and cultural contacts between societies and civilizations. They are present across the African continent where free-range scaveng ingvillage chickens are found in all agroecological zones, ranging from villages in the humid and sub humid tropical rain forests of West and Central Africa to the temperate highlands of East Africa and

the arid and semi-arid regions of the Sahel and Kalahari deserts (Dagris 2007). With an estimated total population of 1.6 billion at the end of 2010 (Faostat, 2012), they are the most abundant livestock species in Africa, contributing to a significant part of the continent's agricultural economy. Although the African continent is rich in galliform species, the recognized main wild ancestor of domestic chickens, the red jungle fowl Gallus gallus, is endemic to sub-Himalayan northern India, southern China and Southeast Asia (Delacour 1977), where the putative centers of domestication of the species are present (Tixier- Boichard *et al.*, 2011). Therefore, domestic chickens, though abundant on the continent, are an introduced species from Asia. Whether or not the species was domesticated in a single geographic area in Southeast Asia (Fumihito *et al.*, 1994) or across the geographic range of the wild ancestor in both South and Southeast Asia (Kangina kudru *et al.*, 2008) is still disputed today. Opinions among scholars are divided concerning when the species arrived and the routes by which chickens entered and dispersed across the continent (Gifford- Gonzalez and Hanotte 2011). Archaeological data are patchy while molecular genetic information is just starting to emerge the initial trigger for the adoption of domestic chickens by African communities could have been socio cultural and/or recreational(e.g., cock fighting)rather than as a new source of food. Textual records indicate that chickens were already present in

Egypt by the time of the Third Dynasty of Ur (c. 2,113–2,006 BC), and the oldest recognizable occurrence of the bird in art dates from the second half of the fourteenth century BC. Houlihan and Goodman (1986) suggest, however, that the first evidence for its consumption comes from the tomb of Petosiris, which dates to the fourth century BC, though this suggestion has been challenged by (Mac Donald and Edwards 1993).

2.3 History of poultry In Sudan:

Poultry keeping in the rural areas of Sudan is one of the most ancient household activities which are practiced in both transhumant and in settled life areas. A family usually keeps a variable number of birds, from local breeds, around the homestead and no distinct system of poultry management is followed. The birds are kept free around the house compound and use the same shelter as that utilized by the family. The importance of village poultry keeping in the Sudan, as a factor contributing to the nutritional level of the family, is fully realized. Therefore, efforts are being made to promote poultry production under village conditions and to control diseases. These efforts were started by the establishment of demonstration units at provincial veterinary Head quarters, educational centers and at agricultural pumping schemes. Then, a model poultry farm was established in Khartoum North, with the objective of providing good quality hatching eggs, graded cockerels and extension services to village poultry keepers. An advisory programme

was also implemented to deal with the breeding, housing, feeding and management aspects of poultry production. Despite the government's efforts, no improvement has been made in the rural poultry production and the official attention has attracted commercial intensive poultry production and research work for improving the production of local breeds under an intensive system. In nearly all African countries, poultry production in the rural areas is predominantly based on a free-range system utilizing indigenous types of domestic fowl (Kitalyi, 1998). The system is characterized by a family ownership of the birds. The birds are then left to scavenge in order to meet their nutritional needs. The feed resources vary depending on the local conditions and the farming system. Housing may not be provided and even if it is provided, local materials are usually used (Atunbi and Sonaiya, 1994). Management is very minimal with some variations of gender roles in the activities (Achiempong, 1992). The health of the birds is not guaranteed because there are no disease control programmers. The birds are exposed to many disease conditions. Among other, the Newcastle disease has been noted as the most prevalent and devastating poultry disease in many African countries (Chrysostome *et al*., 1995). Parasites are also prevalent due to favorable conditions (Permin and Hansen, 1998). It was concluded that the major constraints affecting the rural poultry production are Newcastle disease and parasites, inadequate housing and poor feed supplementation, especially in the dry season (Illang *et al*.,

2000). Women have important responsibilities in the rural poultry production in the two zones.(Sayda *et al* 2012)

2.4 Salmonella and *E. coli* in food system:

The shelf life of low-moisture foods like nuts may be a year or more. Thus, outbreaks associated with these products often span many months. Cases of salmonellosis associated with consumption of raw almonds were reported over periods of 8 and 9 months (Isaacs *et al*., 2005) , and peanut butter– associated outbreaks were reported over 5 and 9 months . The duration of these outbreaks supports laboratory evidence that Salmonella can survive for long periods of time in almonds, pecans, peanut butter, and walnuts (Beuchat, *et al.,* 2007). *Escherichia coli* illnesses have been epidemiologically associated with consumption of in shell hazelnuts (Centers for Disease Control and Prevention. 2011) and walnut kernels (Canadian Food Inspection Agency. 2011.); these outbreaks occurred months after the product was harvested.

handlers may store untreated nuts for 12 months or longer in controlled environments (between 4 and 20 C) or at ambient temperatures. Ambient temperatures are common during shipping and retail handling (Danyluk, *et al.,* 2006) . After purchase, consumers may store nuts for up to an additional 12 months in the freezer, refrigerator, or at ambient temperatures (Lee *et al.,* 2011). In general, microbial populations on nuts or in nut products remain unchanged at refrigerator or freezer

temperatures, whereas storage at room temperature and above leads to slow but steady declines of Salmonella (Martha kimber *et al* 2012).

2.5 Growth and survival of *Salmonella* and *E. coli* :

Sprout producers have recently been faced with several *Salmonella enterica* and *Escherichia coli* outbreaks. Many of the outbreaks have been traced to sprout seeds contaminated with low levels of human pathogens. Alfalfa seeds were inoculated with *S.enterica* and *E. coli* strains isolated from alfalfa seeds or other environmental sources and sprouted to examine growth of these human pathogens in association with sprouting seeds. *S. enterica* strains grew an average of 3.7 $\log_{10}$ on sprouting seeds over 2 days, while *E. coli* strains grew significantly less, an average of 2.3 $\log_{10}$. The initial *S.enterica* or *E.coli* inoculum dose and seed-sprouting temperature significantly affected the levels of both *S. enterica* and *E. coli* on the sprouts and in the irrigation water, while the frequency of irrigation water replacement affected only the levels of *E. coli* . Colonization of sprouting alfalfa seeds by *S. enterica* server Newport and *E. coli* strains transformed with a plasmid encoding the green fluorescent protein was examined with fluorescence microscopy. *Salmonella* server Newport colonized both seed coats and sprout roots as aggregates, while *E. coli* colonized only sprout roots.

Sprout producers in North America, Asia, and Europe have faced numerous *Salmonella* and *Escherichia coli* outbreaks since 1995. Several of the outbreaks were traced to sprouts grown from seeds

contaminated with low levels of human pathogens (Ponka *et al,*.1995). As a result of the many recent outbreaks traced to sprouts and guidance by a U.S. Food and Drug Administration document (Anonymous. 1999), many producers now sanitize their sprout seeds with 20,000 ppm of calcium hypochlorite before sprouting and test each crop of sprouts for *S. enterica* and *E. coli* O157:H7. Epidemiological evidence suggests that this sanitation protocol may prevent some, but not all, outbreaks and experimental sanitation of naturally contaminated alfa lfa seeds did not eliminate *S. enterica* from the seeds (Stewart, *et al* 2001). Reported that no seed sanitation method has been shown to eliminate *S. enterica* or *E. coli* O157:H7 from laboratory-contaminated seeds. In addition, the recommended calcium hypochlorite method does not kill or remove all naturally occur ring non pathogenic bacteria from alfalfa seeds, suggesting that bacteria on seeds may be in locations inaccessible to calcium hypochlorite treatment. (Lang, *et al.*, 2000)

Sprouts present an unusual food safety predicament compared to other fresh produce because bacteria, including *S. enterica* and *E. coli* , may multiply by several logs on the sprouting seeds during the first few days of germination . Experiments with radish and alfalfa sprouts grown from laboratory-contaminated seeds have demonstrated that human pathogens may be present between plant cells inside sprouts, where they can resist decontamination treatments . Washing sprouts in water only slightly reduces the number of bacteria found on the sprouts, and green

sprouts are usually not cooked before being eaten. Thus, sprouts are a good vehicle for food-borne pathogens (Andrews *et al.*, 1982).

2.6 Transmission and Infection of *Salmonella* and *E. coli :*

Human salmonellosis is associated with *Salmonella* in poultry. The distribution of different serotypes is unequal in humans and poultry, and the different serotypes in poultry are probably not equally pathogenic for humans . Nevertheless, considering the relationship between humans and poultry, all phases in the poultry production chain, including the farm, should seriously control *Salmonella.* Two major epidemiological processes determine the *Salmonella* status of a flock at a certain moment. The first process is the introduction of the pathogen into the flock. This introduction can occur either vertically or horizontally. At vertical introduction, the infection enters the flock via the hatchery. The second process that determines the prevalence in a flock is the extent of transmission from infected chickens to susceptible flock-mates. The most important route of transmission of *Salmonella* to another animal is the fecal–oral route, where feces contaminated substances are pecked from the environment. The susceptibility of an individual broiler and the amount of infectious agent at exposure are important for a successful infection Thus, the transmission in a flock depends on the susceptibility of the chickens and the infection pressure.(Heres *et al.,* 2003).

Avian colibacillosis caused by is one of the prime causes of morbidity, mortality and decrease in productivity associated with heavy

economic losses to the poultry industry, by its association with various disease conditions, either as primary or as a secondary pathogen. It affects birds of all ages. Faeco-oral route is the main route of infection following ingestion of contaminated feed and water. Intestinal tract of animals, including poultry, is the most important reservoir of *E. coli*. Transmission of pathogenic *E. coli* through egg is common and can result in huge mortality in chicks. Pathogenic coliforms are more frequent in the gut of newly hatched chicks than in the eggs from which they hatched suggesting rapid spread after hatching. The most important source of egg infection seems to be fecal contamination of the egg surface with subsequent penetration of the shell and membranes. Coliform bacteria can be found in litter and fecal matter. Pathogenic serotypes can also be introduced into poultry flocks through contaminated well water (Heres *et al.,* 2003).

2.7 Age dependence:

Data presented indicate a profound effect of age at initial exposure on the persistence of infection and a lesser effect on the development of effective immunity to re-challenge. The percentage of birds positive for *Salmonella* was high until 8-9 weeks of age, regardless of the age at which the birds were infected (1, 3 or 6 weeks). The birds infected at 3 and 6 weeks of age produced a more rapid and higher antibody response (IgY and IgA) than those infected at one week of age, but in all cases infection persisted for a considerable period despite the

presence of high antibody levels. Following a re-challenge infection with S. Typhimrium, all three previously-infected groups had fewer bacteria in the gut , spleen and liver compared with age-matched birds receiving a parallel primary infection. However, the birds primary infected at 3 and 6 weeks of age cleared infection more rapidly than those infected at a younger age. Interestingly older-primed birds had higher specific T lymphocyte proliferative responses and specific circulating levels of IgY antibody at time of re-challenge. Although birds initially infected at1 week of age and those that were previously uninfected produced a stronger antibody response following re-challenge, they were slower to clear *Salmonella* from the gut than the older-primed groups which expressed a stronger T lymphocyte response. The data presented indicate that clearance of *Salmonella* from the gut is age-dependent and the propose that this relates to the increased competence of the enteric T cell response. The findings that *Salmonella* persists beyond 8-9 weeks, irrespective of age at exposure, has implications for the broiler sector and indicates the need to remain *Salmonella* free throughout the rearing period. Moreover, the re-challenge data demonstrates that infection at a young age is less effective in producing protective immunity than in older chickens. This feature of the development of protective immunity needs to be considered when developing vaccines for the broiler sector of the poultry industry.(Beal *et al*,. 2004).

2.8 Housing and Environment factors:

As in other husbandry fields, the aim in chicken production is to obtain the yield in a desirable level at the lowest cost. As the chickens have spent their life in poultry houses, in order for the chicken to be able to perform their yield capacities entirely, they should be kept in a good environment conditions with a good care as well as genetic features. An adequate environment within poultry houses is a very important requirement for success in the poultry industry. In poultry houses environmental conditions mean physical (heat, humidity and air movement) and chemical factors (ammonia and carbon dioxide in the compound of the air). Chickens and their wastes in poultry houses generate different forms of air pollution, including ammonia, carbon dioxide, methane, hydrogen sulfide and nitrous oxide gases, as well as dust (Kocaman *et al*., 2005).

Gases such as carbon dioxide, ammonia and methane may accumulate and reach toxic levels if adequate ventilation is not maintained. These different air pollutants may cause risk to the health of both chickens and farm workers. Poor environments normally don't cause disease directly but they do reduce the chickens' defenses, making them more susceptible to existing viruses and pathogens (Quarles and Kling, 1974).

Aerial ammonia in poultry facilities is usually found to be the most abundant air contaminant. Ammonia concentration varies depending upon several factors including temperature, humidity, animal density

and ventilation rate of the facility. Chickens exposed to ammonia showed reductions in feed consumption, feed efficiency, live weight gain, carcass condemnation, and egg production (Reece and Lott, 1980). Humidity and temperature also have an impact on air quality. Ventilation is an important consideration for controlling. heat, humidity and different gases. In the poultry houses This research was conducted to determine the effects of laying hen, optimal temperature is required up to 15- environmental factors (ammonia, carbon dioxide, 20 c°. Environmental temperature was correlated with hydrogen sulfide, dust, temperature, relative humidity) on many measures of performance including feed and egg production, feed consumption and feed conversion water consumption, body weight, egg production, feed ratio. conversion, and egg weight (Sterling *et al*., 2003). The reduction of egg production under heat stress may have been related to the altered respiratory pattern. In case of reduction of environmental temperature, they consume much feed in order to maintain their body heat. Studies on the effects of dust in animal housing generally indicate potential for adverse effects on the healthy, growth and development of animals (Janni *et al*., 1985; Feddes *et al*., 1992). Reparable aerosol particles with in poultry housing have been shown to decrease bird growth (Butler and Egan, 1974), increase disease transfer within flocks, and increase condemnation of meat at processing plants (Simensen and Olson, 1980). In poultry houses of laying hen, optimal relative humidity should be

between 60-70%. In case of low relative humidity, dust has increased, and in addition to this, the respiratory diseases in the chickens have been seen (Bahar Kocaman *et al*,. 2006)

2.9 Environment sampling and detection of *salmonella spp* and *E. coli* :

Indeed, clinical diagnosis of *E. coli* infection is made by either culture of the organisms from stool samples or a significant antibody titer to O157 LPS in the patient's serum (Besser *et al.,* 1993). Rapid and simple food-screening methods could limit or prevent the release of virulent fecal pathogen-contaminated foods when food handling or processing techniques fail. Most of the well-known traditional methods of virulent pathogen detection are presumptive and indirect, thus relying on detection of fecal coliform bacteria, which are merely indicators of fecal contamination rather than specific indicators of particular virulent enteric pathogens. In drinking water monitoring, techniques such as membrane filtration onto eosin-methylene blue agar or lactose broth culturing in the most-probable-number assay are slow (24 to 48 h) and the results can be ambiguous (Collins *et al.,* 1998).

The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service has developed a series of both presumptive and confirmatory tests for detection of *E. coli* and pathogenic *Salmonella* species (Sharar, *et al* 1995, and U.S. Department of Agriculture 1995). The presumptive tests involve culturing 25g of homogenized meat

samples, while the confirmatory tests entail biochemical and functional tests as well as immunoassays. In these testing schemes, assay speed is sacrificed in favor of assay sensitivity and definitive identification, making such testing impractical for routine, large-scale food industry use. While obviously slow, the USDA's approach is virtually definitive and extremely sensitive, having a sensitivity of #3 CFU/g of food . This extreme sensitivity is probably necessary to detect the small numbers of *E. coli* thought to initiate disease (Szabo *et al*., 1990). PCR and restriction fragment mapping identification techniques can be definitive and extremely sensitive (Samadpour *et al*., 1990) but require hours of processing time and expertise in molecular biology. Enzyme-linked immunosorbent assay (ELISA) can be rapid (one hours), but the speed of antigen binding to micro titer well surfaces is diffusion limited (Stenberg *et al.,* 1988). A potential disadvantage of ECL, however, is that naturally occurring electron carriers, possessing redox potentials equivalent to that of Ru (bpy) 321 or tripropylamine (TPA), may interfere with the ECL redox reaction by either increasing background light or competing with Ru (bpy) 321 and TPA for electrons. To somewhat offset this potential problem, the signal-to-noise ratio can be controlled through the anodic voltage potential, thus optimizing detector sensitivity even in compete competitive redox environments. Also, IMS can be performed prior to introduction of samples into the IM-ECL detector to wash away potentially competing redox components. Use of

the IM-ECL method may obviate preenrichment in some cases, since extremely sensitive detection may be achieved in less than an hour (Gatto-Menking *et al.,* 1995). Although IM-ECL detection and identification must be considered a rapid presumptive screening process, as would any immunoassay for virulent enteric pathogens, suspect samples are purified by IM capture and can be retained for microscopic observation ,biochemical testing, DNA analysis, or other verification techniques. The present work evaluates the utility of the IM-ECL method for assay of *E. coli* O157:H7 and *Salmonella typhimurium* in food and environmental water samples by use of a commercial IM-ECL sensor . The sensor is capable of sequentially assaying 50 samples with an ECL read time of approximately 2 min per sample. In addition, cross-reactivity with other *E. coli* strains is assessed. The commercial *E. coli* antiserum used has been shown to allow capture of 48 to100% of *E. coli* O157 bacteria but was not found to be H7specific (Okrend, *et al* ., 1992). Although the assays presented may not be completely specific for the target enteric pathogens, that shortcoming lies with the nature of the antibodies used. The present work does, however, illustrate the potential of the IM-ECL method for rapid and facile presumptive industrial screening of foods and other samples(Yu and Bruno, 1996).

2.10 Control of *Salmonella* and *E. coli* :

E. coli and *Salmonella* sp. may colonize the gastrointestinal tract of chickens. *E. coli* and *Salmonella* are major food borne pathogens

associated with processed poultry and may cause severe illness and even death in humans (Tauxe, 1991). The presence of *E. coli* on processed poultry is an indicator of fecal contamination. Antibiotics have been used extensively in animal feed to inhibit the growth of intestinal pathogens. However, the continued feeding of antibiotics at sub-therapeutic levels has created concerns about the extent to which usage increases the

possibilities of antibiotic residue, the development of drug-resistant bacteria, and a reduction in the ability to cure bacterial infections in humans (Jensen, 1998).Increased awareness of the potential problems associated with the use of antibiotics has stimulated research efforts to identify alternatives to their use as feed additives. Probiotics (direct-fed microbial) have been suggested as alternatives to the use of antibiotics in food animals. According to Fuller (1989) probiotics are characterized as live microorganisms (e.g., including bacteria, fungi, and yeast) that when ingested by animals have beneficial effects in the prevention and treatment of diseases (Miles and Bootwalla, 1991). The probiotic bacteria must be able to colonize the gastrointestinal tract, survive the low pH of the stomach and bile acids in the intestines, and compete against other microorganisms in the gastro- intestinal tract. The most commonly used probiotics contain strains of lactic acid producing bacteria (e.g., *Lactobacillus*, *Bifidobacterium* and *Streptococcus*), and of these, lactobacilli are the most studied group (Balevi *et al*., 2001). Lactic

acid bacteria have been demonstrated to inhibit the *in vitro* growth of many enteric bacteria, including *Salmonella typhimurium and E. coli*, and have been used in both humans and animals to treat a broad range of gastrointestinal disorders. reported that the major metabolites of lactic acid bacteria, short chain fatty acids (SCFA) and lactic acid are responsible for their antimicrobial activity against *E.coli* in the intestine(Murry *et al.*, 2004).

Chapter Three

Materials and Methods

3.1 Study area:

The study was carried out in Khartoum state which has desert and semi-desert climate. The state receives little infrequent rain with an average less than 300mm per year, and is characterized by three distinct seasons; cold-dry from November to February, hot-dry from March to June, and hot-wet season from July to October; and wide diurnal and annual temperature variations. The highest mean maxima occur during March-May, ranging between 35-43^{o}C and the lowest mean minima occur during December-February ranging between 15-28^{o}C (Adel and Omer 1999).

3.2 Collection of samples:

A total of 108 samples were collected randomly from the parent houses in Khartoum state at age 25 , 35 and 45 weeks as followed 36 sample in 25weeks ,36 sample in35weeks and 36 sample in 45weeks from points feeds, water, feathers, nests, litters and coloaca aseptically.

3.3 Bacteriology:

All samples were cultured in Nutrient Broth, XLD agars and MaConkey's agars, for the growth of microorganisms Biochemical tests

were performed for identification of the isolates (Abdalla and Siham 2009). The total viable count (TVC) of the isolated microorganisms was carried out according to the method of miles and misra (1938)

3.4 Cultural Methods: as follows :

3.4.1 Viable count at 37c° :-

The total viable count (TVCs) of the isolated microorganism was carried out according to the method of Harrigan and MacCance (1976). A serial dilution of each sample was made to form (10^{-1} up to 10^{-5}).

3.4.2 Serial dilutions:

Five small, sterile test tubes were prepared labeled (1) through (5) and then 9 ml of distilled water was added to each test tube. One ml of the original solution was pipetted into test tube number one. Bacterial suspension was mixed thoroughly (using the vortexes on each bench) before proceeding to the next step. one ml of the diluted bacterial suspension from the first test tube was withdraw into the second test tube using a clean pipette . Continual in this fashion until serial dilution of original bacterial suspension into test tube number five was made. In first test tube, the bacteria was diluted 10 fold, a 1:10 or 1 x 10^{-1} dilution, in test tube five was the bacteria diluted from the original tube to obtain a 1 x 10^{-5} dilution. Each dilution was spread out on a disposable Petri-dish contained plate count agar the hockey stick was used to spread the bacterial suspension evenly over the entire surface of

the plate. Each dilution was cultured in duplicates. The plate was allowed to dry. This process was done with the remainder of the bacterial dilutions.

All the plates were taped together and incubated, upside down, at 37 ^{0}C for 24 hours. . Plates with 20-300 colonies were counted, then the average number of colonies was multiplied by dilution factor to give the number of colonies forming unit per ml and then multiplied by ten to give the number of colonies forming unit per ml of the culture.

3.4.3 Bacterial Isolation and Identification:-

Isolation and identification of bacteria were done based on their morphological, staining, cultural, hemolytic and biochemical properties. For isolation of aerobic bacteria the samples were inoculated onto blood agar plates and incubated at 37 ^{o}C for 24 hours. for purification of different types of colonies were sub-cultured on fresh blood agar. For isolation of *E.coli* the samples were inoculated on MacConkey agar whereas XLD agar *salmonella spp* .

3.4.3.1 Nutrient Agar

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.

3.4.3.2 Blood Agar

Blood agar was prepared according to Barrow and Felltham, (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.

3.4.3.3 MacConkeys Agar:

MacConkeys agar contains peptone 20 grams, lactose 10 grams, bile salts 5 grams, neutral red 0.075 grams and agar 12 grams. PH as 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.

3.4.3.4 Xylose –Lysine Deoxycholate Agar (XLD-Agar):

This was prepared by Suspend they 56,68 grams in 1000 ml distilled water . heated with frequent agitation until the medium boils .(not autoclaved or over heated).

Identification was based mainly on the followings;

i/ Gram stain (ii) Microscopic and macroscopic examination of morphology (iii) Haemolysis on blood agar (iv) Indole production (v) Presence of catalase (vi) Acid and gas production . The methods TVC and Identification of the different strains that used were as described by Harrigan and MacCance, (1976). Barrow and Felltham, (1993).

3.4.4 Gram Staining and Microscopy:-

Gram stain was used to study shape of isolation bacteria. A sterile loop was used to carry single colony to prepare a suspension in one drops of normal saline on clean slide.

A thin smear was made, dried in air and fixed by flaming .All slides prepared were placed on a rack. Then the staining was carried out using the standard Gram staining procedure where the slides were flooded with crystal violet stain for 30sec and washed with water. The slides were then covered with Lugol's iodine solution for 30sec and washed with running tap water. The stain was decolorized by using 70% alcohol this was carried out until the stain stopped running. The slide were then counterstained with dilute 10% carbol fuchsin for 30 secand and washed by running tap water.

The stained slides were dried by blotting on filter paper and examined by the light microscope under (1000x) magnification using oil immersion lens.

3.4.5 Indole Test:-

Peptone water medium was inoculated with test culture and incubated at 37Cº for 48 hours .One ml of Kovac's reagent was run down the side of the tube where a pink ring appears on the reagent layer within a minute in positive samples.

3.4.6 Catalase test:-

The test was carried out as described by barrow and Felltham (1993) a drop of 3 % aqueous solution of hydrogen peroxide was placed on a clean slide. A colony under test on nutrient agar was picked off and mixed, using the wire loop with the drop of hydrogen peroxide. The test is considered positive if gaseous bubbles are observed.

3.4.7 Sugars Fermentation tests:-

The peptone water sugar was prepared as described by Barrow and Felltham (1993) and was inoculated with test culture. The inoculated culture, in test tube, was incubated and examined daily. Reddish color indicated acid production and gas production was indicated by developing of an empty space in the Durham’s tube.

Chapter four

Results

Table 1: Comparison of mean total viable count of bacteria log_{10} CFU/cm^2 ± Sd at different operational points: In 25 weeks old parent chicken (Descriptive Statistics):

Source	Mean log_{10} CFU/cm^2	Std. Deviation
Water	6.10	.09
Coloaca	6.16	.04
Litter	6.22	.07
Nest	6.25	.07
Feed	6.23	.10
Feather	6.23	.04

As shown in Table 1 the total viable count was of highest contamination in nests (6.25±0.07) this was followed by feed and feather (6.23) and litter (6.22) and Coloaca (6.16) but low contamination was in water (6.10±**0.09)** at age 25 weeks.

Table 2: Comparison of mean total viable count of bacteria log_{10} CFU/cm^2 ± Sd at different operational points: In 35 weeks old parent chicken (Descriptive Statistics):

Source	Mean log_{10} CFU/cm^2	Std. Deviation
Water	6.15	.10
Coloaca	6.25	.08
Litter	6.19	.06
Nest	6.09	.11
Feed	6.16	.05
Feather	6.17	.10

As shown in Table 2 the total viable count was of highest contamination in coloaca (6.25±0.08) this was followed by litter (6.19) and feather (6.17) and feed (6.16) and water (6.15) but low contamination was in nests (6.09**±0.11)** at age 35 weeks.

Table 3: Comparison of mean total viable count of bacteria log_{10} CFU/cm^2 ± Sd at different operational points: In 45 weeks old parent chicken (Descriptive Statistics):

Source	Mean log_{10} CFU/cm^2	Std. Deviation
Water	4.09	0.17
Coloaca	6.28	.06
Litter	6.13	.07
Nest	6.07	.15

Feed	6.21	.12
Feather	6.12	.18

As shown in Table 3 the total viable count was of highest contamination in coloaca (6.28±0.06) this was followed by feed (6.21) and litter (6.13) and feather (6.12) and nest (6.07) but low contamination was in water(4.09±**0.17)** at age 25 weeks.

Table 4: Number and percentage of bacteria isolated and identified in different operational points from parent chicken in 25 weeks :

Source	***E. coli***	**Salmonella**	**Total**
Water	6(100%)	0(0.0%)	6(16%)
Coloaca	6(100%)	0(0.0%)	6(16.6%)
Litter	0(0.0%)	6(100%)	6(16.6%)
Nest	0(0.0%)	6(100%)	6(16.6%)
Feather	0(0.0%)	6(100%)	6(16.6%)
Feed	0(0.0%)	6(100%)	6(16.6%)
Total	12(33.3%)	24(66.6%)	36(100%)

Isolation and identification of bacteria at different operational points under investigation revealed the present of only 2 species of bacteria (Table 4) *Escherichia coli* was isolation from water and cloaca

(6 isolate each) and *Salmonella* was isolation from litter and nests *and* feather and feed (6 isolate each).

Table 5: Number and percentage of bacteria isolated and identified in different operational points from parent chicken in 35 weeks :

Source	***E .coli***	**Salmonella**	**Total**
Water	0(0.0%)	6(100%)	6(16%)
Coloaca	6(100%)	4(66.6%)	10(27.7%)
Litter	5(83%)	1(16.6%)	6(16.6%)
Nest	2(33%)	4(66.6%)	5(13.8%)
Feather	0(0.0%)	6(100%)	6(16.6%)
Feed	0(0.0%)	6(100%)	6(16.6%)
Total	12(33.3%)	27(75%)	39(100%)

Isolation and identification of bacteria at different operational points under investigation revealed the present of only 2 species of bacteria as shown in (Table 5) *Escherichia coli* was isolation from cloaca (6 isolate) and from litter (5 isolate) and from nest (2 isolate) but *Salmonella* was isolation from water ,feed and feather(6 isolate each) and from cloaca and nest (4 isolate each).

Table 6: Number and percentage of bacteria isolated and identified in different operational points from parent chicken in 45 weeks :

Source	***E. coli***	**Salmonella**	**Total**
Water	4(66.6%)	0(0.0%)	4(66.6%)
Coloaca	6(100%)	0(0.0%)	6(16%)
Litter	5(83%)	4(66.6%)	9(25%)
Nest	5(83%)	5(83%)	10(27.7%)
Feather	6(100%)	3(50%)	9(25%)
Feed	6(100%)	3(50%)	9(25%)
Total	32(88.8%)	15(41.6%)	47(100%)

Isolation and identification of bacteria at different operational points under investigation revealed the present of only 2 species of bacteria as shown in (Table 6) *Escherichia coli* was isolation from cloaca, feather and feed (6 isolate each) and from litter, nest (5 isolate each) and from water (4 isolate) but *Salmonella* was isolation from water ,feed and feather(3 isolate each) and from nest (5 isolate) and from litter (4 isolate).

Chapter Five

Discussion and Conclusion

Food-borne illnesses in human beings due to bacterial pathogens and their toxins are well documented worldwide. In this study the total viable count was high in the nests (6.25±0.07) but was low contamination in water (6.10±**0.09)** at age25 weeks. The TVC was high in coloaca (6.25±0.08) but was low contamination in nests (6.09±**0.11)** at age 35 weeks, The TVC high in coloaca (6.28±0.06) but was low contamination in water (4.09±**0.17)** at age 45 weeks. But other study by Nasrin *et al* **(**2007) reported that the mean TVC obtained from litter, and drinking water was higher (37.0±1.79)× 10^5 and (31.33±1.12)× 10^5 CFU/ml but feed was lower (6.5±1.87)× 10^5 CFU/gm, respectively. The litter samples revealed the highest TVC which might be due to the presence of elevate number of bacterial population in gut and high contamination of *E. coli* and *salmonella spp*. .

Compared to the present study, many authors found a higher prevalence of *Salmonella* in other developing countries: 51.2% in Argentina, 68.2% in Ethiopia, and 72% in Thailand. In developed countries, the levels of *Salmonella* contamination in chicken ranged from 15 to 70% and the average value was about 35% , 16% in Ireland ,22% in the USA,36.5% in Belgium and 55% in Spain(Cardinale , Perrier 2003). Even if the serotypes isolated vary geographically, *Salmonella* has been frequently isolated from chickens throughout the

world dominguez *et al* (2002). In Spain, Jorgensen *et al* (2002). in the United Kingdom, and Roy *et al* (2002). In the USA showed that *Salmonella* was one of the most prevalent servers in chicken products.

More research was focused on the influence of the housing system on environmental *Salmonella* contamination. The analysis of the existing data of an EU-wide baseline study on *Salmonella* in laying hens flocks performed in 2004-2005 showed a significant difference in *Salmonella* prevalence according to housing type. In cage systems the highest *Salmonella* prevalence was found followed by a intermediate prevalence in barn systems and the lowest prevalence in the free range systems. More than 51% of all *Salmonella* isolates were serotype as *Salmonella* enteritis. (Anon *et al*2008).

The risks of rodents regarding *Salmonella* persistence in poultry houses have been evaluated in only a few studies: in broiler breeder and layer breeder houses in the UK13, 26 and in layer houses in the USA.31–33,35 Persistence studies have not yet been reported for organic livestock farms. Chances of *Salmonella* persistence on farms are about two times higher when rodents are encountered by farmers. The presence of *Salmonella* in the litter of broilers decreases as the number of flocks raised on it increases Roll *et al* 2011.

Conclusion:

Poultry meat contamination with microorganisms which cause deterioration in food quality, and especially those which cause food borne diseases, is a major challenge for poultry industries in Sudan must aim at improving hygiene control during farm , the improvements on the hygienic situation could only be obtained by intervention at several places in the poultry farm at the same time.

Recommendations :

Bio-security system should be done in poultry farms for improvement of hygienic situation and production of clean food for our health .

We recommend following the bio- security system in a way good at poultry farms .We recommend people clean foods well before use to maintain our health and our lives.

References:

Abdalla M.A , Siham E. Siuman and Alian Y.Y.H.A 2009 , Microbial Contamination of Sheep Carcasses at Modern SlaughterhdPse in Khartoum State , *sud. J.Vet. Sci. Anim. Husb. Vol*. 48(1&2).

Achiemong, C. K. (1992). Women in poultry keeping for sustainability in Ghana. In Proceedings, 19th World Poultry Congress, Amsterdam, the Netherlands, 20-24 Sept. 1992 (1992), pp. 71-78.

Adel E , and Omer k. (1999). Geological Research Authority, Khartoum1999/Sudan, www.Geospatial world.com.

Andrews W. H , Mislivec P. B , Wilson C. R , Bruce V. R , Poelma P. L , Gibson R , Trucksess M. W and Young K . 1982. Microbial hazards associated with bean sprouting. *J. Assoc*. Off. Anal. Chem. **65:**241-248.

Anon Newsletter , Safehouse, Rescape, Immerseel Ed, Van F and Cepero Y, Nys R , Yangüela, Lidon J, and Hernandis M. D. (2008). Calidad del huevo en jaulas enriquecidas, *Conference proceeding - XXXVII Symposium Sec. Esp. WPSA, I Congreso Internacional de Sanidad y Producción Animal*, Noviembre 2000,Barcelona, Spain, 61-80.

Anonymous. 1999. Guidance for industry: reducing microbial food safety hazards for sprouted seeds and guidance for industry: sampling

and microbial testing of spent irrigation water during sprout production. Fed. Regist. **64:**57893-57902.

Atunbi O. A , and Sonaya, E. B. (1994). An assessment of backyard poultry housing in Osogbo, Osun State, Nigeria. *African Network for Rural Development Newsletter, 4*, 7.

August K , Skog K , Jagerstad M , and Steineck G . 2004 . Assessment of the human exposure to heterocyclic amines. *Carcinogenesis* 18(10):1931-1935.

Bahar Kocaman1, Nurinisa Esenbuga , Ahmet Yildiz , EkremLaçin and Muhlis Macit 2006. Effect of Environmental Conditions in Poultry Houses on the Performance of Laying Hens**.** International Journal of Poultry Science 5 (1): 26-30,

Balevi T, U.S. An, Coskun B, Kurtoglu. V and Etingul I.S , 2001. Effect of dietary probiotic on performance and humoral immune response. Br. Poult. Sci.,. 42:456-461.

Beal RK , Wigley P, Powers C, Hulme SD, Barrow PA, Smith AL.2004**.** Age at primary infection with Salmonella enterica serovar Typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge**.** *Vet Immunol Immunopathol.* Aug;100(3-4):151-64.

Besser R. E, Lett S. M , Weber J. T, Doyle M. P, Barrett T. J, Well J. G, and Griffin P. M . 1993. An outbreak of diarrhea and hemolytic

uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA **269:**2217–2220.

Beuchat L. R , and Mann D. A . 2010. Factors affecting infiltration and survival of Salmonella on in-shell pecans and pecan nutmeats. J. Food Prot. 73:1257–1268.

Beuchat L. R and E. K. Heaton. 1975. Salmonella survival on pecans as influenced by processing and storage conditions. Appl. Environ. Microbiol. 29:795–801.

Butler E.J. and Egan B.J. 1974. Undirectional air flow isolators: a review. World's Poult. Sci., 30: 32-41.

Cardinale E , Perrier Gros-Claude J.D, Tall F, Cissé M, Guèye E.F, and Salvat G. 2003. Prevalence of Salmonella and Campylobacter in Retail Chicken Carcasses in Senegal .Revue Élev. Méd. *vét. Pays trop*. 56 (1-2) : 13-16.

Centers for Disease Control and Prevention. 2011. Investigation update: multistate outbreak of E. coli O157:H7 infections associated with in-shell hazelnuts. Available at: http://www.cdc.gov/ecoli/2011/ hazelnuts0157/index.html. Accessed 12 May 2011.

Chrysostome C. A. M., Bell J. G, Demey F and Verhulst A. (1995). Seroprevalences to three diseases in village chicken in Benin. *Prev. Vet. Med.*, *22*, 257-261.

Collins C. H, Lyne P. M, and Grange J. M. 1989. Microbiological methods, 6th ed., p. 133–140. Butterworth-Heinemann Publishing,

Oxford. Family poultry production in Africa. Morogoro, Tanzania 4th–8th 2000.

Croxen M. A and Finlay B. B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. *Nat Rev Microbiol* **8**, 26-38.

Dagris (2007). Domestic Animal Genetic Resources Information System (DAGRIS). (Eds. S. Kemp, Y. Mamo, B. Asrat and T. Dessie). International Livestock Research Institute, Addis Ababa, Ethiopia.

Danyluk M. D, A. R. Uesugi, and L. J. Harris. 2005. Survival of Salmonella Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. J. Food Prot. 68:1613–1622.

Danyluk M. D , Harris L. J , and Schaffner D. W. 2006. Monte Carlo simulations assessing the risk of salmonellosis from consumption of almonds. J. Food Prot. 69:1594–1599.

Delacour J. (1977). The pheasants of the world (2nd ed.). Hindhead: Spur Publications.

Faostat and FAO Statistical Yearbook 2012. FAO, Rome, Italy. http://faostat.fao.org/. Accessed August 23, 2012.

Feddes J.J.R , Koberstein B.S , Robinson F.E and Ridell C , 1992. Misting and ventilation rate effects on air quality, and heavy from turkey performance and healty. Can. Agri. Eng., 34: 177-181.

Fumihito A , Miyake T, Sumi S , Takada M , Ohno S , & Kondo N. (1994). One subspecies of the red jungle fowl (Gallus gallus gallus) suffices as the matriarchic ancestor of all domestic breeds. Proceedings

of the National Academy of Sciences of the United States of America, 91, 12505–12509.

Gatto-Menking D. L, H. Yu J. G, Bruno M. T, Goode M, Miller and Zulich A. W. 1995. Sensitive detection of biotoxoids and bacterial spores using an immunomagnetic electrochemiluminescence sensor. Biosens. Bioelectronics**10:** 501–507.

Gifford-Gonzalez D and Hanotte O. (2011). Domesticating animals in Africa: Implications of genetic and archaeological findings. Journal of World Prehistory, 24, 1–23.

Gordon D. M and Cowling A. (2003). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. *Microbiology* **149**, 3575-3586.

Heres l , Urlings h. a. p, Wagenaar j. a and jong m. c. m. de. (2003). Transmission of Salmonella between broiler chickens fed with fermented liquid feed. *Epidemiol. Infect*. , 132, 107–116. *f* 2004 Cambridge University Press.

Hazariwala A , Sanders Q , Hudson C.R , Hofacre C , Thayer S.G and Maurer J.J. (2002) Distribution of staphylococcal enterotoxin genes among *Staphylococcus aureus* isolates from poultry and humans with invasive staphylococcal disease. *Avian Dis* 46(1):132-136.

Houlihan P. F and Goodman S. M. (1986). The birds of ancient Egypt. Warminster: Aris and Phillips Ltd. Insoll, T. (1994). Preliminary results

of excavations at Gao, September and October 1993. Nyame Akuma, 41, 45–48.

Illango J, Etoori A, Olupot H and Mabonga J (2000). *Rural poultry production in two Agroecological zones of Uganda*. Paper presented at the Second Research Coordinated meeting on

Isaacs S, Aramini J, Ciebin B, Farrar J. A, Ahmed R, Middleton D, Chandran A. U, Harris L. J, Howes M, Chan E, Pichette A. S, Campbell K, Gupta A, Lior L. Y, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, and Ellis A. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of Salmonella Enteritidis. J. Food Prot. 68: 191–198.

Ishii S , Ksoll W. B , Hicks R. E , and Sadowsky M. J . (2006). Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Appl Environ Microbiol* **72**, 612-621.

Jansen .W, Notermans S, Wernars K, Soentoro P. S and Dufrenne J,.1996. DNA-hybridization and latex agglutination for detection of heat-labile and Shiga-like toxin-producing Escherichia coli in meat. Int. *J. Food Microbiol*.13:31–40.

Janni K.A , Redig P.T , Mulhausen J.R and Newman J.A , 1985. Turkey grower barn monitoring results. ASAE, St. Joseph, MI., 85: 4527-4532.

Jensen, B.B 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. Anim. Feed Sci., 7: 45- 64.

Jorgensen F , Bailey R , Williams S , Henderson P , Wareing D. R , Bolton F.J , Frost J. A, Ward I , Humphrey T.J , 2002. Prevalence and numbers of Salmonella and Campylobacter spp. on raw, whole chickens in relation to sampling methods. Int. J. Food Microbiol., **76**: 151-164.

Kanginakudru S , Metta M , Jakati R. D and Nagaraju J. (2008). Genetic evidence from Indian red jungle fowl corroborates multiple domestication of modern day chicken. BMC Evolutionary Biology, 8, 174. doi:10.1186/1471-2148-8-174.

Kaper J. B , Nataro J. P , Mobley H. L. (2004). Pathogenic *Escherichia coli*. *Nat Rev Microbiol* **2**, 123-140.

Kiranmayi Ch, Bindu and Krishnaiah N.2010. Detection of Escherichia coli O157:H7 prevalence in foods of animal origin by cultural methods and PCR technique**.** *Veterinary World, Vol*.3(1):13-16

Kitalyi, A. J. (1998). Village chicken production systems in rural Africa. Household food security and gender issue, FAO Animal Production and Health Paper 142. Rome, Italy. pp. 160.

Kocaman B , Yaganoglu A.V and Yanar M . 2005. Combination of fan ventilation system and spraying of oil-water mixture on the levels of dust and gases in caged layer facilities in Eastern Turkey. J. Appl. Anim. Res., 27: 109-111.

Kocaman B, Yaganoglu A.V and Yanar M. 2005. Combination of fan ventilation system and spraying of oil-water mixture on the levels of

dust and gases in caged layer facilities in Eastern Turkey. J. Appl. Anim. Res., 27: 109-111.

Lang M. M, Ingham B. H , and Ingham S. C. 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* O157:H7 from alfalfa seeds prior to sprouting. *Int. J. Microbiol*. **58:**73-82.

Lee L. E, Metz D, Giovanni M and Bruhn C. M. 2011. Consumer knowledge and handling of tree nuts: food safety implications. Food Prot. Trends 31:18–27.

MacDonald K. C and Edwards D. N. (1993). Chicken in Africa: The importance of Qasr Ibrim. Antiquity, 67, 584–590.

March S. B and Ratnam S. 1989. Latex agglutination test for detection of *Escherichia coli* serotype O157. J. Clin. Microbiol. **27:**1675–1677.

martha a. kimber, harbir kaur, luxin wang, michelle d. danyluk, and linda j. harris1 .2012. Survival of Salmonella, Escherichia coli O157:H7, and Listeria monocytogenes on Inoculated Almonds and Pistachios Stored at 219, 4, and 24uC. Journal of Food Protection, Vol. 75, No. 8, 2012, Pages 1394–1403.

Miles R.D and Bootwalla S.M. 1991. Direct-fed in animal production. In: Direct-fed Microbials in Animal Production. A Review, National Food Ingredient Association, West Des Moines Iowa, USA, pp. 117-132.

Murry A.C , Hinton A and Morrison H .2004. Inhibition of Growth of *Escherichia coli*, *Salmonella typhimurium*, and *Clostridia perfringens* on Chicken Feed Media by *Lactobacillus salivarius* and *Lactobacillus plantarum.* International Journal of Poultry Science 3 (9): 603-607.

Mwacharo, J.M , Nomura K, Hanada H , Jianlin H , Hanotte O and Amano T. (2007). Genetic relationships among Kenyan and other East African indigenous chicken. *Animal Genetics, 38*, 485–490.

Nasrin M.S, Islam M.J, Nazir K.H.M.N.H, Choudhury K.A. and Rahman M.T .2007. Identification of bacteria and determination of their load in adult layer and its environment. *J. Bangladesh Soc. Agric. Sci. Technol., 4(1 & 2): 69-72.*

Okrend A. J. G., B. E. Rose and Lattuada C. P. 1992. Isolation of *Escherichia coli* O157:H7 using O157 specific antibody-coated magnetic beads. J. Food Prot. **55:**214–217.

Permin, A and Hansen J. W. (1998). Diagnostic methods. In *Epidemiology, diagnosis and control of poultry parasites, FAO Animal Health Manual*. Rome 72-115.

Ponka A, Andersson Y, Sitonen A, de Jong B, Jahkola M, Haikala O, Kuhmonen A, and Pakkala P. 1995. *Salmonella* in alfalfa sprouts. Lancet 345:462-463.

Power M. L , Littlefield-Wyer J , Gordon D. M , Veal D. A and Slade M. B . (2005). Phenotypic and genotypic characterization of

encapsulated *Escherichia coli* isolated from blooms in two Australian lakes. *Environ Microbiol* **7**, 631-640.

Quarles C.L and Kling H.F. 1974. Evaluation of ammonia and infectious bronchitis vaccination stress on broiler performance and carcass quality. Poult. Sci., 53: 1592-1596.

Reece F.N and Lott B.D. 1980. The effect of ammonia and carbon dioxide during brooding on the performance of broiler chickens. Poult. Sci., 59:1654-1661.

Rindhe S.N, Zanjad P. N, Doifode V.K, Siddique A. and Mendhe M.S.2008. Assessment of microbial contamination of chicken products sold in Parbhani city. *Veterinary World, Vol.1(7)*: 208-210.

Roll V. F. B , Dai Pra M. A , and Roll A. P 2011. Research on *Salmonella* in broiler litter reused for up to 14 consecutive flocks. *Poultry Science* 90 :2257–2262.

Roy P , Dhillon A.S , Iauerman H , Schaberg D.M , Bandli D , Johnson S. 2002. Results of Salmonella isolation from poultry products, poultry, poultry environment, and other characteristics. Avian Dis., **46**: 17-24.

Samadpour M, Liston J, Ongerth J. E and Tarr P. I. 1990. Evaluation of DNA probes for detection of Shiga-like toxin-producing *Escherichia coli* in food and calf fecal samples. Appl. Environ. Microbiol. 56**:**1212–1215.

Savageau M.A. (1983). *Escherichia coli* habitats, cell types, and molecular mechanisms of gene control. *Am Nat* 122, 732-744

Sayda A, Ali M, Mohammed A. Bakheet and Abeer E. ElNazeer. (2012) .Rural Poultry Keeping in South Gezira, Sudan. Pertanika J. Trop. Agric. Sci. 35 (3): 569 - 580 .

Sharar A. K and Rose B. E .1995. United States Dept. of Agriculture, Food Safety and Inspection Service: protocol for isolation and Identification of *Escherichia coli* O157:H7. Revision 4 of laboratory communication 38. U.S. Department of Agriculture, Washington, D.C.

Simensen E and Olson L.D. 1980. Aerosol transmission of pateurella multocida in turkeys. Avian Diseases., 24: 1007-1010.

Stenberg M and Nygren H. 1988. Kinetics of antigen-antibody reactions at solid-liquid interfaces. J. Immunol. Methods **113:**3–15.

Sterling K.G., Bell D.D, Pesti G.M and Aggrey S.E 2003. Relationsships among strain, performance, and environmental temperature in commercial laying hens. J. Appl. Poult. Res., 12: 85-91.

Stewart D. S , Reineke K. F, Ulaszek J. M and Tortorello M. L .2001. Growth of *Salmonella* during sprouting of alfalfa seeds associated with salmonellosis outbreaks. J. Food Prot. **64:**618-622.

Szabo R , Todd E, Mackenzie J, Parrington L, and Armstrong A. 1990. Increased sensitivity of the rapid grid membrane filter enzyme-labeled antibody procedure for *Escherichia coli* O157 detection in foods and bovine feces. Appl. Environ. Microbiol. **56:**3546–3549.

Tauxe R.V. 1991. Forward: transmission of human bacterial pathogens through poultry (Banquet , address). In *Colonisation Control of Human Bacterial Enteropathogens* ed. Blankenship, L.C. pp.

Tenaillon O , Skurnik D , Picard B and Denamur E. (2010). The population genetics of commensal *Escherichia coli*. *Nat Rev Microbiol* 8, 207-217.

Tixier-Boichard M, Bedd'hom B and Rognon X. (2011). Chicken domestication: From archaeology to genomics. Comptes Rendus Biologies, 334, 197–204.

U.S. Department of Agriculture. 1995. Food Safety and Inspection Service: procedure for isolation of *Salmonella* from food. Laboratory communication no. 75. U.S. Department of Agriculture, Washington, D.C.

Walk S. T , Alm E. W , Calhoun L. M , Mladonicky J. M , Whittam T. S. (2007). Genetic diversity and population structure of *Escherichia coli* isolated from freshwater beaches. *Environ Microbiol* **9**, 2274-2288.

Yu H and Bruno J G.1996. Immunomagnetic-electrochemiluminescent detection of Escherichia coli O157 and Salmonella typhimurium in foods and environmental water samples .*Journals .asm.org. applied and environmental microbiology*, pp. 587–592**.**

Yu H, Bruno J. G, Cheng T, Calomiris J. J, Goode M. T and Gatto-Menking D. L. 1995. A comparative study of PCR product detection

and quantitation by electro chemiluminescence and fluorescence. J. Biolumin. Chemilumin. **10:**239–245.