Introduction

Poultry refer to number of domesticated avian species which include the chicken (reared for laying eggs"-layer or meat production - broiler)-turkey- duck and other water fowls –game bird which have different type of production (EPA,2007)

Poultry provide globally important sources of animal protein and are amongst the most intensively reared of all livestock species (Damerow, 1994).

Biosecurity can be refer to measures taken to prevent or control the introduction and spread of infectious agent to a flock, such as infectious agents, whether they cause clinical or subclinical disease (Australia biosecurity manual; 2009)

Biosecurity procedures should be implemented with objective of preventing the introduction and dissemination of infectious agents in the poultry production chain. Biosecurity will be enhanced with the adoption and implementation of Good agricultural practice and the Hazard Analysis Critical Control Point HACCP(OIE 2015).

Adequate biosecurity measure can improve overall flock health, cut the cost of treatment reduced the losses and improve farm profitability (Mrigen, 2006).

The Poultry meat consumption was increased in the few years (FAO,1999)

The concept of assuring the safety of food derived from animals from the moment of birth of the animal until the products derived from it reach the consumer dictates the need for biosecurity implementation throughout the value chain. This often expressed from farm to fork, (OIE ,2015).

HACCP is food production, storage, and distribution monitoring system for identification and control of associated health hazard aimed at prevention of contamination instead of end product evaluation (Tompkin, 1990). HACCP strategies identify the areas where pathogens may enter the system, ways to eliminate them and the methods to show that the chain of production is being

continually and consistently audited. This is achieved by dissecting every procedure in the production chain (Tompkin, 1990).

HACCP is food management system which concentrate prevention strategies of known hazard and the risk occurring at specific point in the food (Shmoury 2000)

HACCP can be applied throughout the food chain from primary production to final consumption and its implementation should be guided by scientific evidence of risk to human health .As well as enhancing food safety ,implementation of HACCP can provide other significant benefit .In addition ,the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety , (CAC/1 1969,REV ,4/2003 Annex).

In last year's Sudan start poultry industry for local consumption and then for export to achieve that by prevent diseases and good management then have safe food. Khartoum State produces almost 90% of Sudan's poultry production.

Objectives:

1 - To evaluate the situation of poultry farms in Khartoum state and to application biosecurity in poultry farms.

2 -To know the possibility to apply HACCP system in biosecurity of poultry farms.

3- To apply the good hygiene practices GHP .

Chapter One
LITERATURE REVIEW

1-1-History of poultry in Sudan

History of the poultry industries in the Sudan began in 1926, by entering 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 1958was 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.

Diseases continue to impart the world's poultry industry and health problems of flock is part of poultry industry. Some of these can be easily controlled, while other is causing more reason for concern. However proper feeding, housing vaccination disinfection and hygiene are essential modes of disease control in the management of all form of livestock farming and poultry particularly when kept under intensive system. In the strict sense, disinfection consists of destroying disease – production microbe se.g. Viruses, bacteria, protozoa and fungi, by chemical and physical mean if spores are killed during the process, only then is it said to be sterilization, (Mergien, 2006)

1-2-Biosecurity in poultry farm

With the advent of intensive farming multi ages flock are reared in close system in some region, which can encourage the spread of variety of pathogens. Unless the challenges from the pathogens are controlled through strict management practices, vaccination and medication cannot adequately protect the flock (Teresa, 2001).

Biosecurity procedures should be implemented with the objective of preventing the introduction and dissemination of infectious agents in the poultry production chain .biosecurity will enhanced with the adoption and implementation of the principles of good agricultural practices and the Hazard Analysis Critical Control Points (HACCP) (OIE, 2015) biosecurity procedures in poultry production . According to the manual of Commonwealth of department of Agriculture (Australia, 2009) it is important to account all factors that may impact on the biosecurity of the production area .this factors should include the species of bird being produced ,location and layout of the property and production area , source of water supply disease status of district proximity to other production area with avian species and type of wild life , personnel and vaccination

1-2-1Who is involved in biosecurity?

Biosecurity involves many different of stakeholder at national level. Government agencies have primary interest but industry, scientific research institutes, specialist interest group and the nongovernmental organizations and general public all have role to play (FAO, 2006)

Biosecurity requires the adoption of asset of attitude and behavior by people to reduce risk in activities involving poultry production and marketing for that it must be clear that even comprehensive biosecurity plan can not completely eliminate the possibility of disease, but it can significant reduce the possibility of disease entering farm (FAO, 2O11)

1-2-2 The basic biosecurity principles for poultry sector include

1-2-2-1 Isolation

Disease can be brought on to the farm by people ,new poultry , equipment , village poultry or wild animals including wild bird it is important to restrict access to birds wherever possible . for the best practice the farmer must restrict access to the entire farm by fence the farm and then build another fence or arrange the area where

poultry are kept so people will know when they are about enter the poultry area . Whenever possible, the all –in all-out single age group should be used

According to (Clark,2006) poultry farms should be constructed as isolated from other animal facilities as is possible. The rule has been 1-3 miles from any other poultry facilities. In many of poultry producing state this has been difficult to implement the facilities should be constructed so that they can be easily repair to keep bird in and wildlife out. (OIE, 2015) Also refer to other animal of the appropriate (resident) species and age, should not be permitted access to poultry houses, no animals should have access to other building, such as those used to store feed, eggs or other material. Dead bird should be removed from poultry houses as soon as possible and disposed of in safe and effective method (OIE, 2015) Poultry are allowed to range out door, feeders, feed and other items may attract wild birds should be kept indoors and. Poultry should not be allowed to access to source of contamination, such as household waste, litter storage area other animals stagnant water and water of unknown quality. The nesting area should be inside the poultry house

1-2-2-2- Sanitation and disinfection program in poultry farm

Sanitation refers to the quality of cleanliness, while disinfection refers to the reduction of pathogen. Reducing the load of pathogens in the environment of the flock will decrease the risk of disease. As stated earlier, disinfectants are chemical agent that kill pathogen on contact whereas cleaning prior to disinfection exposes the pathogen to the disinfectants (Jeffrey, 2005).

Microbiological contamination can be prevented and controlled by using proper management practices and health care products such as disinfectant(MUS,2008) The main purpose of disinfectant use is to reduce the number of pathogen agent in environment so the potential for diseases occurrence in poultry farm is reduced(Block,2001)

1-2-2-3 To keep poultry farm clean the farmer must

- Once each day, clean all bird areas and equipment by using appropriate disinfectant, at the recommended concentration for the recommended contact time
- Protection the feed from pets and moisture by keeping it in close containers also cleanup the feed spills as soon as discover
- Changing all litter after every change of flock and regularly whenever it becomes wet.
- Cleaning and repair the entire poultry houses between flock.
- Implement effective pest control program.

In addition of these all personnel and visitor entering a poultry house should wash their hand with soap and water or sanitize them using disinfectant. Personnel and visitors should also change foot wear, use boot spray or use property maintained disinfectant foot bath .the disinfectant solution in the foot bath should be change regular basis to ensure its efficacy (OIE, 2015)

The drinking water supply to poultry houses should be potable according to world Health Organization or to the relevant standard and microbiological quality must be monitored to any suspect contamination also the water delivery system should be cleaned and disinfected between flock when the house empty also the equipment should be cleaned and sanitized before being taken to poultry house.

Container should be cleaned and disinfected between each use, or disposed in safe method they recommended to heat treated feeds with or without the addition bactericidal or bacteriostatic if it not possible use of the bactericidal or bacteriostatic is recommended (OIE, 2015).

1-2-2-4 Traffic control

Controlling of traffic flow in and out of susceptible areas to limit exposure. This would include fencing, gates, human and vehicle control within the farm and into the farm; notifying the visitor that flock areas are out of bound to outside visitor;

controlling the movement of equipment and products to and from the farm (Msami 2007) To minimize stress poultry should be transported in well ventilated container and should not be over crowded, exposure to extreme temperatures should be avoided (OIE, 2015)

1-2-2-5 Vaccination

As it was stated in the FAO animal production and health manual (2006) vaccination as a support strategy may be considered when the disease has spread to such an extent that it has overwhelmed the resources of disease control authorities or the economic cost of widespread slaughter camping cannot be borne. It can also be considered at an earlier stage when veterinary service infrastructure and capacities prove to be very weak and insufficient to curb the spread of disease .FAO and OIE have made recommendation for the use of OIE –approved vaccines these vaccines provide excellent protection against clinical disease in chickens by reducing mortality and production losses.

1-2-3 Biosecurity program

According to (Clarke, 2006) steps taken by production team implementing a biosecurity program includes define objective (Example goal – free from any bacterial diseases)

- Agree on controls –define and identify potential sources of these organisms
- Establish standard operating procedures –these should be farm specifics, with sufficient details required for future training.
- Document by self-audit- record sources and status of stocks terminal hygiene vaccine administration, rodent control program and visitor' log
- Undertake statistically valid monitoring of effectiveness.
- Review flock status eg depletion-problem may mean standard operation procedure need further development or objective need modification
- Review objective

Biosecurity begin with the physical layout of the farm and the production cycle. Production sites should be isolated from other production facilities so if problems occur. Spread is minimized. Site with feed mill, breeders, broiler, rendering plants slaughter houses and hatcheries offer some economics in organization but makes implementation of effective biosecurity very difficult (Gillinsky, 2006)

1-2-4 Management to prevent disease in poultry house

The more important physical principles of disease prevention include favorable geographic location of the farm in respect to other proper location of buildings in relation to each other and to prevailing wind currents, proper design of the building inside and out, and design and positioning of equipment. Long-range planning and programming of the operation, whether large or small, is very important and should consider movement patterns of various vehicles and equipment, work traffic of regular and holiday caretakers and special work crews, feed delivery and storage, and the system for moving eggs and flocks from the farm. An avian pathologist can be helpful in avoiding some common pitfalls, but to avoid high-risk disease situations, consultation should be done when the farm is being designed and the production programmed, rather than after it is developed and serious trouble is evident. Developing and achieving adoption of biosecurity measures will require multidisciplinary and participatory approach working with producers intermediaries ,live bird market trader and ,for back yard poultry ,communities (Guerne et al .,2009) Because of the fact that livestock production, disease occurrence and traditions differ between countries, it is likely that biosecurity also differs between countries, it is therefore important to investigate biosecurity routines in different regions and population (NÖremark et al., 2010)

1-3 HACCP

HACCP (pronounced "Hassip") stands for the Hazard Analysis and " Critical Control Point system .It allows predication of potential risk to food safety to

prevent them before happen. By using HACCP, seller will no longer have to rely solely on routine inspections to spot potential food safety hazard (price et al, 1993)

The term "hazard analysis and critical control point (HACCP) " was first introduction in the European Directive 93\ 43\CE(1993) (Betrolini et al., 2007)

According to (Mortimore 2001), HACCP can be define as a " common sense " approach to food safety management.

The hazard analysis and critical control point (HACCP) system is a food safety management strategy which has been widely tested, and established as an effective means of preventing food borne diseases where correctly implemented (WHO, 1993 and CAC, 1993) it is being promoted internationally as the preventive system of hazard control that is considered to the most effective and efficient way to ensure food safety (FA0, 1995)

End product testing alone is unable to assure safe food production and hence the hazard analysis critical control point (HCCP) approach has been adopted for the elimination or reduction of the identified hazard to an acceptable level (Walker. et al. ,2003) . This preventive system is designed for the safe production of food by applying control at any point in production where hazard occur or where previously introduced hazards can be controlled before consumption.

The application of HACCP based on technical and scientific principles to produce safe food. An ideal application of HACCP would include all processes in food production from farm to the table .The food industry has embraced HACCP as the best system for preventing food safety problems (Olson and Slack, 2006)

The main idea behind HCCP is that possible to identify potential hazards and faulty practices at an early stage in food production, processing or preparation. These can then be controlled in order to prevent or minimize risk to health of the consumer or economic loss from food spoilage .HCCP involve the identification of hazards associated with any stage of food production, processing or preparation,

the assessment of related risks, and the determination of steps where control is critical to achieving (NACMCF, 1992)

Any HACCP system is capable of accommodating change such as an equipment design and technology development (CAC, 1997)

HACCP strategy identifies hazards associated with different stages of preparation and handling, assesses the relative risk and identifies point where control measures would be effective (Bryan, 1988;Ehiri et al.,2001).

To successfully implement HACCP in the food supply, authorities responsible for food safety must first be aware of the need to move to a system such as HACCP. Until this need is acknowledged, it is unlikely that commitment at any level can be expected (WHO, 1995).

WHO has recognized the importance of the HACCP System for prevention of food borne disease for over 20 years and has played an important role in its development and promotion. The recent and growing concern about food safety from public health authorities, food industry and consumer worldwide has been the major impetus in application of the HACCP system (WHO, 2007)

1-3-1 Historical overview

The concept of pre-HACCP is attributed to Deming ,who developed in 1950s the leading theory of Total Quality Management system (TQM) (Charisis, 2004) First the Japanese tested this system with great success. In between, the TQM system paved and prepared the way for appearance of an almost full-developed HACCP system in 1960s . The original acronym HACCP was conceived in 1959 and developed by Pillsbury Company, National Aeronautics and Space Administration (NASA), World Health Organization \Mediterranean Zoonoses Control Program and U.S.

Army laboratory at Natick, in order to ensure the safety of astronauts' food in 1973 the Pillsbury Company published the food safety through the Hazard Analysis and

Critical Control Point System, which was the first document on HACCP concept and techniques (Charisis, 2004).

Twenty years later, this system was internationally recognized and accepted for food safety assurance, including, not only microbiological safety of food stuffs but also chemical and physical hazard. Since then and for many years, HACCP system have been applied on a voluntary basis in many food industry (Charisis, 2004)

1-3-2 HACCP and food safety

HACCP is a system to identify and prevent the potential food safety problem with the manufacture, distribution and use of food product.

Microbial hazard analysis attempt to identify the pathogen in row material, routes for pathogen to enter the processing environment, the methods for their elimination, and potential problems with the finished product when not handled appropriated, Risk is an estimation of how likely a potential hazard could result in problem. The severity of the resulting food safety problem is inversely related to the level of risk acceptable (Baker, 1995)

Identification of preventive measures which eliminate or limit the risk from potential hazard is primary goal of HACCP plan development.

Preventive measures are implemented at critical control point (CCP) where a potential hazard associated with a food material, environmental location, manual procedure or mechanical process can be controlled. CCP parameters are characterized by a critical limit and target value bounded by tolerance limit, within which the desired level of control is obtained a critical limit separates acceptable from unacceptable. Implementation of a HACCP system require that CCP are monitored, HACCP plan require that processing records are maintained, documenting that processing procedures were diligently implemented (Baker,1995)

1-3-3Advantages of HACCP (FAO\1995):-

- The HACCP system as it applies to food safety management uses the approach of controlling critical point in food handling to prevent food safety problem.
- The system which is science based and systematic, identifies specific hazards and measure for their control to ensure the safety of food ,HACCP is based on prevention and reduces the reliance on end –product inspection and testing
- The HACCP system can be applied through the food chain from the primary product to the consumer .Beside enhancing food safety, other benefits of applying HACCP are the more effective use of resource savings to the food industry and more timely response to food safety problems
- HACCP enhances the responsibility and degree of control at the level of the food industry. A properly implemented HACCP system lead to greater involvement of food handler in understanding and ensuring food safety, thus providing them with renewed motivation in their work, implementing HACCP does not mean undoing quality assurance procedures of good manufacturing practices already established by company : it does, however, require revision of these procedures as part of systemic approach and for their appropriate integration into the HACCP plan.
- The application of the HACCP system can aid inspection by food control regulatory authorities and promote international trade by increasing buyer confidence
- HACCP system should be capable of accommodating change, such as advances in equipment design, changes in processing procedures or technological development (FAO, 1995)

1-3-4 Definition

Much term are used in discussion of HACCP that must be clearly understood to effective develop and implemented a plan.

The following definition are provided by CAC (2001)

1-3-4-1 Control (verb):

To take necessary action to ensure and maintain compliance with critical established in the HACCP plan

1-3-4-2 Control (noun):

The state wherein correct procedures are being followed and criteria are being met.

1-3-4-3 Control measure:

Any action and activity that can be used to prevent or eliminate a food safety hazard or reduced it to an acceptable level.

1-3-4-4 Control action:

Any action to be taken when the result of monitoring at the CCP indicate a loss of control.

1-3-4-5 – Critical control point (CCP):

A step at which control can be applied and is essential to prevent or eliminate a food safety hazard o reduce it to an acceptable level.

1-3-4-6 Deviation:

Failure to meet a critical limit

1-3-4-7- Flow diagram:

A systematic representation of sequence of step or operation used in the production or manufacture of a particular food item.

1-3-4-8-HACCP:

A system which identified, evaluate, and control hazard which are significant for food safety.

1-3-5-1 Other definition (FDA, USDA and NACMCF, 1997):

1-3-5-1-CCP decision Tree:

A sequence of questions to assist in determining whether a control point is a CCP

1-3-5-2 Critical limit:

A maximum and\or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of food safety hazard.

1-3-5-3 HACCP plan:

The written document which is based upon the principle of HACCP and which delineates the procedures to be followed.

1-3-5-4-HACCP Team:

The group of people who are responsible for developing, implementing and maintaining the HACCP system.

1-3-5-5 Hazard:

A biological, chemical or physical agent that is reasonably likely to cause illness or injury in the absence of its control.

1-3-5-6- Hazard Analysis:

The process of collecting and evaluating information on hazard associated with the food under consideration to decide which are significant and must be addressed in the HACCP plan.

1-3-5-7-Prerequisite programs:

Procedures include good manufacturing practices that address operational condition providing the foundation for the HACCP system

1-3-5- 8 Monitor:

To conduct a planned sequence of observations or measurement to assess whether a CCP is under control and to produce an accurate record for future use in verification.

1-3-5-9 Severity:

The seriousness of the effect(s) of hazard.

1-3-5-10 Step:

A point, procedure, operation or stage in the food system from primary production to final consumption.

1-3-5—11 Validations:

That element of verification focused on collecting and evaluating scientific and technical information to determine if the HACCP plan, when properly implemented, will effectively control the hazards.

1-3-5-12 Verification:

Those activities other than monitoring, that determine the validity of the HACCP plan and that system is operating according to the plan.

1-3-6-Principle of the HACCP system:

In 1972 Pillsbury Company in the US began the application of its HACCP concept to the manufacture of its consumer food products. This primordial HACCP system consisted of three principle conduct hazard analysis, determine critical control point and establish monitoring procedures (Sperber, 2005).

The modern HACCP system is built upon seven principles, HACCP principle start with hazard assessment then include critical control point (CCPs) determination, establishment of critical limit establishment of methods of monitoring CCPs corrective action and procedures of verification and end with effective record system (Jay et al., 2000)

1-3-6-1- Conduction of hazard analysis

The Codex Hazard Analysis and Critical Control Point (HACCP) system and guideline for its application (1997) define a hazard as A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect the hazard analysis is necessary to identify for the HACCP plan which hazard are of such nature that their elimination or reduction to acceptable level is essential to the production of a safe food. An inaccurate hazard analysis would inevitably lead to the development of an inadequate HACCP plan.

Thus Hazard analysis requires technical expertise and scientific background in various domains for proper identification of all potential hazards. Knowledge of food science and HACCP is necessary for the performance of satisfactory hazard analysis

A Hazard is any property that may cause an unacceptable health risk to customer. Hazard may be biological, chemical or physical.

- Chemical hazards include toxin heavy metals and improperly used pesticide, cleaning compound and food additives
- Biological hazards include harmful bacteria, viruses or other microorganism
- Physical hazards include foreign object that may cause illness or injury for example metal glass, plastic and wood

When hazard analysis determination, the primary potential food safety risk are determined at each stage of the preparation process. Each food- preparation process has its own potential safety hazards. These hazards may vary from deli to deli and from recipe to recipe (Price et al., 1993).

The process of conducting a hazard analysis involves two stages. During the first, hazard identification; the HACCP team reviews the ingredients used in the product, the activities conducted at each step in the process and the equipment used, the final product and its method of storage and distribution, and the intended use and consumers of the product. Based on this review, the team develops a list of potential biological, chemical or physical hazards which may be introduced, increased, or controlled at each step in the production process (FDA, USDA and NACMCF, 1997)

The probability that a hazard will occur is called a risk. The risk may take a value from zero to one depending on the degree of certainty that the hazard will be absent or that it will be present. After hazard identification, a hazard analysis must be conducted to understand the relative health risk to man or animal posed by

hazard. It is a way of organizing and analyzing the available scientific information on the nature and size of the health risk associated with the hazard. The risk may have to be assessed subjectively and simply classified as low, medium, or high. Only those hazards considered by the HACCP team to present an unacceptable risk of being present are taken forward to stage 7, Principle 2(FAO, 2001).

Once a food safety hazard has been identified then appropriate control measures should be considered. These are any action or activity that can be used to control identified hazard, such that it is prevented, eliminated or reduced to an acceptable level. The control measure may also include training of personnel for particular operation, covered by GAP, GMP and GHP (FAO, 2001)

1-3-6-2 Determination of Critical Control Point (CCP)

A critical control point, which is the control, can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. The potential hazard must be addressed in determining CCPS (FDA, USDA and NACMCF, 1997)

Complete and accurate identification of CCPs is fundamental to controlling food safety hazards. The information developed during the hazard analysis is essential for the HACCP team in identification which steps in the process are CCPs. One strategy to facilitate the identification of each CCP is the use of a CCP decision tree (Appendix 1) although application of the CCP decision tree can be useful in determining if a particular step is a CCP for a previously identified hazard; it is merely a tool and not a mandatory element of HACCP. ACCP decision tree is not substitute for expert knowledge (FDA, USDA and NACMCF,1997). Critical control points are located at any step where hazard can be prevented, eliminate or reduce to acceptable level. CCPs must be carefully developed and documented (Appendix 2). In addition they must be used only for purposes of product safety. (FDA, USDA and NACMCF, 1997)

1-3-6-3- Establishing of critical limit:

A critical limit is a maximum and \or minimum value to which biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard (FDA, USDA and NACMCF, 1997).

A critical limit is used to distinguish between safe and unsafe operating conditions at a CCP. Critical limit should not be confused with operational limit, which are established for reasons other than food safety (FDA, USDA and NACMCF, 1997).

Each CCP will have one or more control measure to assure that the identified hazards are prevented, eliminated or reduced to acceptable levels.

Each control measure has one or more associated critical limits. Critical limits may be based upon factors such as temperature, time, physical dimension, humidity, moisture level, water activity, PH titratable acidity, salt concentration, available chlorine, viscosity, preservatives, or sensory information such as aroma and visual appearance. Critical limits must be scientifically based. For each CCP, there is at least one criterion for food safety that is to be met (FDA, USDA and NACMCF, 1997).

1-3-6-4 Establishment of a system to monitor control of the CCP

Monitoring may be physical, microbiological or chemical tests, or by visual or sensory observation. All monitor procedures should be recorded and they should also include the location of the CCP, the frequency of monitoring and satisfactory compliance criteria. Examples include cleaning procedures (what is cleaned how and when it is cleaned, who clean it and with what), temp, hygienic practices, opportunities for cross-contamination and workers illness or infection (Fellow, 2000).

The monitoring of a CCP involves the scheduled testing or observation of a CCP and its limits; monitoring results must be documented. Microbial analyses are not

use to monitor since too much time is required to obtain results. Physical and chemical parameters such as time, pH, temperature, and water activity can be quickly and results obtained immediately (Jay et al., 2005)

1-3-6-5 Establishment of corrective action to be taken when monitoring indicates that particular CCP not under control

These are action to be taken when monitoring indicates a deviation from un established critical limit. The final rule requires a plant's HACCP plan to identify the corrective action to be taken if a critical limit is not met. Corrective action are intended to ensure that product injurious to health or otherwise adulterated as result of deviation enters commerce (FSIS, 1998)

There for corrective action should include the following elements:

- Determine and correction the cause of non –compliance.
- Determine the disposition of non-compliant product
- Recording the corrective action that have been taken

Specific corrective action should be developed in advance for each CCP and included in the HACCP plan (NACMF, 1997).

1-3-6-6- Establishing of procedures for verification to confirm that the HACCP system working effectively

Verification system should be established to ensure that the HACCP system developed for a specific food production system is working effectively to ensure safety to consumers. Both the food producer and the regulatory agency have to be involved in the verification of the effectiveness of the HACCP in the place. Verification methods include testing samples for physical, chemical, sensory and microbiological criteria as established in the HACCP plan (Bibek, 2004).

1-3-6-7 - Establishing of documentation concerning all procedures and record appropriate to these principles and their application

This principle requires the preparation and maintenance of a written HACCP plan by the food establishment. The plan must detail the hazards of each individual or categorical product covered by the plan. It must clearly identify the CCPs and critical limit for each CCP. CCP monitoring and record keeping procedures must be shown in establishment's HACCP plan. HACCP plan implementation strategy should be provided as a part of the food establishment documentation (Anon, 1999).

All HACCP associated activities must be fully documented, results of all tests and monitoring system records, defect or failures noted and remedial (corrective) action documented by well-trained accountable staff (Bell et al.,2005).

1-3-7- Flow diagrams

The development of an HACCP plan for a food establishment begins with the construction of a flow diagram for the entire process. The diagram should begin with the acquisition of row materials and include all steps through packaging and subsequent distribution (Jay et al, 2005).

1-3-8- Application of the HACCP principles (HACCP Plan)

Logic sequence for application of HACCP

1-3-8-1- Assemble HACCP team

The food operation should assure that appropriate product specific knowledge and expertise is available for the development of an effective HACCP plan optimally, this may be accomplished by assembling a multidisciplinary team where such expertise is not available on site, expert advice should be obtained from other sources. The scope of the HACCP plan should be identified. The scope should describe which segment of the food chain is involved and the general classes of hazards to be addressed (e.g. it covers all classes of hazards or only selected classes) (FAO, 1998).

1-3-8-2- Describe product

A full description of the product should be drawn up, including relevant safety information such as; composition physical\ chemical structure (including Aw pH, etc) durability, packaging and storage condition and method of distribution (FAO, 1998).

1-3-8-3-Identify intended use

The intended use should be based on the expected uses of the product by the end user or consumer. In specific cases. Vulnerable groups of the population, e.g. institutional feeding, may have to be considered (FAO, 1998).

1-3-8-4- Construct flow diagram

The HACCP team should construct the flow diagram. The flow diagram should cover all steps in the operation. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specified operation (FAO, 1998)

1-3-8-5- On Site verification of flow diagram

The HACCP team should confirm the processing operating against the flow diagram during all stages and hours of operation and amend flow diagram during where appropriate (FAO, 1998).

1-3-8-6-Identify and analyses hazard(s) – (principle1)

Effective hazard identification and hazard analysis is the key to a successful HACCP plan. All read or potential hazards that may occur in each ingredient and each stage of the commodity system should be considered (FAO, 2001).

Using the flow diagram and the list of potential hazards already in the file, the analyst now needs to evaluate the significance of hazards: (Anon, 2005).

- By checking that no hazard has been overlooked;
- By checking that all hazards identified by using the flow diagram are collated in a summary (tables, list…).

- By checking that calculation of the criticality index (specified on a separate document) provides a valid evaluation and hence a correct rating of risk;
- By checking through the calculation of the criticality index that identified risks are real (and thus excluding any risks with zero index value)

All list of preventive measure, each specific to each identified hazard as applied in the particular establishment, with procedures for implementation must appear in file.

1-3-8-7- Determine Critical Control Points (principle 2)

They may be more than one CCP at which control is applied to address the same hazard the determination of a CCP in the HACCP system can be facilitated by the application of decision tree, which indicates logic reasoning approach .application of decision tree should be flexible, given whether the operation is for production, slaughter, processing, storage, distribution or other. It should be used for guidance when determination CCPs. This example of a decision tree may not be applicable to all situations (Appendix 1). Other approaches may be used. Training in the application of the decision tree is recommended (CAC, 2003).

If hazard has been identified at a step where control is necessary for safety, and no control measure exists at that step, or any other, then the product or process should be modified at that step, or at any earlier or later stage, to include control measure (CAC, 2003).

1-3-8-8 Establishing of critical limits for each CCP – (principle 3)

At each critical control point, critical limit must be established. In some cases, more than one critical limit will be specifying at a particular CCP they must be specific and reasonable for each control measure and for instance can be limits on temperature, time, humidity, moisture level, water activity, pH, titratable acidity, salt concentration, preservation, aroma and visual appearance they can be maximum, minimum, or range value (Omer, 2002).

Critical limits may be deriving from sources such as regulatory standards and guidelines literature survey, experimental studies practical experience and expert advice. Critical limits may exceed a regulatory requirement. In some cases, processing variations may require the use the use of target level to ensure that critical limits are met (Omar, 2002).

1-3-8-9- Establishing a monitoring procedure – (principle 4)

Monitoring is the mechanism for confirming that critical limits at each CCP are being met. The method chosen for monitoring must be sensitive and produce a rapid result so that trained operatives are able to detect any loss of control the step. This is imperative so that corrective action can be taken as quickly as possible so that loss of product will be avoided or minimize (FAO, 2001).

Monitoring can be carried out by observation or by measurement, on samples taken in accordance with statistically based sampling plan. Monitoring by visual observation is basic but gives rapid result, and can be therefore being acted upon quickly. The most common measurement taken are time, temperature and moisture content (FAO, 2001)

1-3-8-10- Establishing of corrective action – (principle 5)

Specific corrective action must be developed for each CCP in the HACCP system in order to deal with deviations when they occur (CAC, 2003).

The action must ensure that the CCP has been brought under control .Actions taken must also include proper disposition of the affected product. Deviation and product disposition procedures must be documented in the HACCP record keeping (CAC, 2003).

1-3-8-11 Establishment of procedures for verification-(principle 6)

Verification requirement should be established and verification should be carried out to confirm that the HACCP system is working according to plan. Verification should concern specifically the control of CCPs, compliance with target and

tolerance, the effectiveness and suitability of monitoring activities and the effectiveness and suitability of preventive and corrective action. During verification, evidence should be gathered to confirm that the plan is suitable for the product and process concerned (Early, 1997).

Modification of the plan and the system may be made according to the result of verification. Verification activities should normally be scheduled although, in the event of unexpected problems arising, unscheduled verification may be undertaken (Early, 1997).

1-3-8-12 Establishment of record – keeping procedures- (principle 7)

Developing of a strict record keeping system that demonstration control over critical control points (Omar, 2002):

- Advice facility management and government official of the performance of A plant's HACCP plan on day –to- day basis.
- Provide evidence of a proper and safe operation.
- Serve as a mechanism for indicating serious problem and assisting the responsible individual(s) in the determination of proper corrective action
- Permit traceability of the product.

The record used and kept in the total HACCP system should be instance:

- Ingredients and packaging materials records (Omar,2002)

  - Supplier certification documentation showing compliance with processor's specifications.
  - Processor audit records verifying supplier compliance.
  - Storage temperature records for temperature sensitive ingredient and for packaging materials.
  - Storage time records of limited shelf life ingredients.
- Records indicating compliance with labeling or sealing specification of packaging materials.

- Records related to product safety:

- Sufficient data and records to establish the efficacy of barriers in maintaining product safety.
- Sufficient data and records to establishing the safe shelf life of the product when the age of the product can affect safety
- Documentation of the adequacy of the processing procedures from a knowledgeable process authority
- Processing records (Omar, 2002)

  -Records from all monitored CCPs.

  -Record verifying the continued adequacy of the processes
- Product storage and distribution records (Omar,2002)
- Temperature records.
- Records showing no product shipped after shelf life date on temperature sensitive product.
- Deviation and corrective action records.
- Monitoring records.
- Verification records.
- Validation records and modification to the HACCP plan, approved revision and changes in ingredients, formulation, processing, packaging and distribution and control, as need.
- Employee training records; and good manufacturing practices records.
- The plant should also keep the HACCP plan records (Omar, 2002):
- List of the HACCP team and assigned responsibilities.
- Description of the product and its intended use.
- Flow diagram for the entire manufacturing process indicating CCPs.
- Hazard associated with each CCP and preventive measure.
- Critical limits.

- Monitoring system.
- Corrective action plans for deviation from critical limits
- Records – keeping procedures for verification and validation of HACCP system and verification data

- **The outcome of Biosecurity using HACCP system**

  The outcome of developing a biosecurity plan using HACCP principle is that there will be greater assurance that flocks are protected against serious disease outbreaks.

  - **5The critical monitoring point identified in Australian code(2001)are:**

- Entry of chicks, litter, equipment, vehicles, people and feed into started pullet farms
- Entry of litter, started pullets, adult fowls, equipment, vehicles, people and feed into egg production farms
- The presence of wild birds and rodents in shed or where hens and pullets range
- Water sanitation farm using surface water for internal shed fogging or bird drinking water for dead birds, reject eggs and manure from the farm
- The presence of non-poultry bird species, other poultry on the farm

1-6 Cause of poultry diseases

This can be divided in to six groups, namely those caused by bacteria, viruses, fungi, parasite, toxins and allergy. There are also those resulting from nutritional deficiencies, poor housing and management and through stress.

1-6-1 Gram-Negative rods

1-6-1-1 Escherichia coli

E. coli is a Gram negative rod facultative rod anaerobic and generally motile organism, E coli strains are in general, non-pathogenic and exist harmless in the intestinal tract of human and animals. Pathogenic E.coli strain cause variety of diseases including gastroenteritis, dysentery, hemolytic uremic syndrome, urinary tract infection, septicemia, pneumonia, and meningitis. However, the major concern in recent years has been the increasing numbers of outbreaks of entero hemorrhagic

E. coli due to consumption of contaminated meat, fruits, and vegetables (Bhanddare, 2008)

1-6-1-1-1 Avian Pathogenic Escherichia coli (APEC)

Avian pathogenic Escherichia coli the causal organism of infection in chickens, are responsible for large economic losses in the poultry industry worldwide this imply any systemic or localized infection cause by E. Coli include many diseases like air sac disease, coli granuloma (Barnes, and Gross,1997). Clinical finding and lesions Concurrent Signs are nonspecific and vary with age, organs involved and 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 fibrin purulent air saculitis ,pericarditis ,perihepatitis ,and lymphocytic depletion of the bursa and thymys.Sporadic lesions include pneumonia, arthritis ,osteomyelitis, peritonitis, and salpingitis(The Merck veterinary manual,2010)

1-6 1-2 Salmonella sp.:-

Salmonellosis has been considered one of the most important infectious disease in both humans and animals(Keusch, 2002).

The widespread occurrence of Salmonella in natural environment and the Intensive husbandry practice used in the meat, fish and shellfish industries has been significant problem in public health (Michael et al., 2007).

Salmonella infections are caused by the ingestion of contaminated food or water, after which the bacteria are able to colonize the small intestine and invade intestinal enterocytes (Jennifer et al., 2003). The most common source of human salmonellosis is food of poultry origin. Human Salmonella infection can lead to several clinical conditions including enteric fever enter colitis and systemic infections (Piyush and Anju, 2008).

Measures should be taken to prevent Salmonella infections by using Salmonella-free feed ingredients, eliminating these pathogens from mixed feed (pelleting), keeping feed clean by good feeding practices and storage facilities, and keeping natural carriers (rodents, wild birds, pets) out poultry feed stock

And houses. Preventing salmonellosis and other types of enteric infections also helps prevent wet droppings, which contribute to wet litter. Above all, eggs should be gathered frequently, especially in the early part of the day when most hens visit the nests. They should be gathered in clean, dry equipment and held in a dry, dust-free area.(Y.M .Saif et al, 2008)12ed

Salmonella prevention and control may be achieved by adopting Good Agricultural Practices and Hazard Analysis Critical Control HACCP and other general measures on hygiene and biosecurity procedures in poultry production OIE (2015)

1-6-2 Gram positive rod

1-6- 2-1Staphylococcus aureus

Staphylococcus.aureus is a gram positive and non- spore forming spherical belong to the staphylococcus genus. The staphylococcus genus is subdivided into 32 species and subspecies. Staphylococcus aureus produces staphylococcal toxin (SE) and it is cause almost of staphylococcal food poisoning (Motvile and Matthews 2008; FDA)

The temperature range for growth of *S.* aureus is 7–48°C, with an optimum of 37°C.

Staphlococcus. Aureus is resistant to freezing and survives well in food stored below -20°C; however,

Viability is reduced at temperatures of -10 to 0°C. *S.* aureusis readily killed during pasteurization or cooking. Growth of *S.* aureus occurs over the pH range of 4.0–10.0, with

An optimum of 6–7 (ICMSF 1996; Stewart 2003).

Staphylococcus. aureus is a facultative anaerobe so can grow under both aerobic and anaerobic

r, growth occurs at a much slower rate under anaerobic conditions (Stewart 2003)

Staphylococcus. Aureus in poultry cause *a*rthritis the disease has been seen in birds ranging from 14 to 70 days of age, but most cases occurred around 35 days old(Mcnamee PT,Smyth, JA.2000). Bacterial arthritis in poultry after septicemia or localized is reported to be associated with Erysipelothrix, Listeria, Mycoplasma, Staphylococcus, and Escherichia (Mohan et al. 2002)

1-6-2- 2 Symptom of disease in human

Staphylococcal food poisoning symptoms generally have a rapid onset, appearing around 1. 3 hours after ingestion (range 1–6 hours). Common symptoms include nausea, vomiting, abdominal cramps and diarrhea. Individuals may not demonstrate all the symptoms associated with the illness. In severe cases, headache, muscle cramping and transient changes in blood pressure and pulse rate may occur. Recovery is usually between 1–3 days (Stewart 2003; FDA 2012).

1-7 - Poultry Production system

Chicken can be reared in different management and production systems Based on chicken length of broodiness, growth rate and number of chicken reared. There are three types of chicken production systems (ANRS BOARD, 2006).

1-7-1- The close system;

In this system poultry production is practices by large companies under controlled environment and advanced managerial standards. They are main source chicken meat.

1-7-2- The semi closed system:

In this system poultry production is practice by medium companies and large private poultry production farms under semi controlled environment and advanced

managerial standards. This system has been introduced recently by medium size companies and private producer.

1-7-3- The open system

All the small, medium and some large poultry farm in Khartoum state are of this type. The farm has open sides houses with gable shaped roofs usually made of corrugated metal. The walls are constructed of bricks and the rest is covered with mesh network. Broiler production in this system is limited, because it is affected by great losses due to high temperature, disease, and low weight gain.

Chapter Tow

Material and Methods

2-1 A study area:

The study data was collected from 5 poultry farms in Khartoum state.

2-2 **Questionnaire**:-

Questionnaire was prepared to get information about the biosecurity system on poultry farm and to assess the situation and application HACCPs in biosecurity of poultry farm.

2-3 Sampling method:-

Sampling was done according to systemic random methods in period of four months from February 2015 to June 2015.

Samples were collected from 5 farms,210 swabs .The swab were taken from 7 sites workers, feeders, walls , water ,drinkers , floor , and feed, duplicated. Transferred in ice box to the microbiology laboratory of collage of veterinary medicine of Sudan university of Science and Technology

2-3-1 Bacteriology:

2-3-2 Isolation and identification:

The isolation and identification was carried out according to Barrow and(Feltham 1993) and (Holt et al, 1994). Organism were obtained from 7 sites of poultry house using preparing MacConkcy agar , Blood agar the plate were incubated at 37ºc for 24 hrs. Well isolated colonies obtained from agar medium and different broth culture of gram positive and gram negative bacteria were constantly sub culture into agar. Slants from time to time incubated at 37 ºc for 24 hrs. and stored in 40ºc. Identification was based on the following: indole production, presence of catalase, hemolysis of blood agar, gram stain.

The samples were examined to isolation bacteria E coli, Salmonella.Sp and Staphylococcus sp.

2-4 Cultural media

2-4-1 Solid culture media

2-4-1-1 Blood agar

As described by Oxoid laboratory products, London (Oxoid Lab)40 grams of the base powder were added to one distilled water. The mixture was then boiled until the powder dissolve completely. The solution was autoclave at 121ºc and 15 pound per square inch for 15 minute; it was then called to 45-50ºc. About 7% of sterile blood was added with gentle rotation and then poured into petri dishes (15-20ml) and left to solidify and kept in refrigerator 4ºc

2-4-1-2 Manitol salt agar :-

Manitol salt agar is differential and selective plate medium used to isolate staphylococcus aureus .

The medium prepared by dissolving 11, 1 g in 100 ml of distilled water

Prepare and sterilize as instructed by the manufacturer. When the medium has cooled to 50-55 C mix well and dispense in sterile Petri dishes. (Cheesbrough ,1999)

2-4-1-3 Xylose Lysine Deoxycholate (XLD) agar:-

XLD agar was used to isolate salmonella and shieglla.Sp species .it is based on xylose fermentation lysine decarboxylation, and hydrogen sulphide production, it is possible to differentiate Salmonella and Shigellae.Sp from most nonpathogenic enterobacteria ,although some faecal commensals such as Proteus species produce identical colonies of the pathogen.

The medium was used at a concentration of 5.3grams in every 100 ml distilled water and Prepared as instructed by the manufacturer the medium was heated with care.

As soon as the medium cooled to about 55 Mix well, and dispense aseptically in sterile Petri dishes date the medium and give it batch number(Cheesbrough ,1999)

2-4-1-4 Eosin Methylene Blue (EMB):-

Eosin methylene blue agar. Slightly selective and differential planting medium for the isolation of gram –negative enteric bacteria (bacilli) isolated from both clinical and nonclinical specimen.

Gram tve bacteria are inhibited. It is widely used for examination of material for the presence of coli forms (Levin, 1918)

The media prepare by suspend 37.5 g of media in 1000ml of dematerialize water , heat to boiling with agitation to completely dissolving and sterile by autoclaving at 121º c for 15 minutes and dispensed into appropriate containers.

2-4-2 Semi solid media

2-4-2-1Motilty medium

The medium was describe by(Cruickshank et al.1975) agar 0.2%was dissolved in nutrient broth and distributed in sterile test tubes containing Craiggie tubes then the media was autoclaved at 121ºc and 15 pounds per square inch.

2-4-2-2 Hugh and Liefson's (O\F) medium

The Hugh and Liefson's medium (Cowan and Steel, 1974) contained pepton ((2g), NaCL,(5g), KHPO4 (0.3G) agar (3g), distilled water (1000ml) and bromocrysol purple, and 0.2% aqueous solution (15ml) the solid were dissolved by heating in the water bath. The PH was adjusted to 7.1 the medium was filtered. The indicator was added. Sterilization was done by autoclaving for 15 minutes and pressure of about 15ib per square inch then sterile glucose (1%) was added to the mixture and they distributed aseptically in ten volumes into sterile test tubes with cotton plugs.

2-4-3 Liquid culture media:

2-4-3 -1 Kligler iron agar (KIA):-

KIA is differential slope medium used in the identification of Salmonella sp ,Shigel sp and other enteric bacteria

KIA reaction is based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide.

-a yellow butt (acid production)and red pink slope indicate the fermentation of glucose only the slope is pink-red due to a reversion of the acid reaction under aerobic condition this reaction is seen with Salmonella and Shigella.Sp species and other enteric pathogen

-Cracks and bubbles in the medium indicate gas production from glucose fermentation. Gas is produced by Salmonella paratyphi and some faecal commensals.

A yellow slope and yellow butt indicate the fermentation of lactose and possibly glucose this occurs with Escherichia coli and other enterobacteria

A red-pink slope and butt indicate no fermentation of glucose or lactose .this is seen with most strain of pseudomonas aeruginosa.

Blacking along the stab line or throughout the medium indicate hydrogen sulphide(H2S) production .

Medium was used at concentration of 5.5 grams in every 100 ml of distilled water.

Used straight wire to inoculate KIA medium, first stabbing the butt and then streaking the slope in azig-zag pattern after inoculation make sure tube tops are left loose (Cheesbroubh ,1999).

2-5 Biochemical test: -

2-5-1 Catalase test:-

This test is used to differentiate bacteria that produce the enzyme catalase, such as staphylococci, from non –catalase producing bacteria such as streptococci

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.

This test was done by putting 2-3 ml of the hydrogen peroxide solution in to tube contain isolation organism

Active bubbling …….positive test catalase produced (Cheesbrough, 1999)

2-5-2 Citrate utilization test:-

This test was one of several techniques used 'to assist in the identification of enterobacteria the test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen.

The test organism was cultured in medium which contained sodium citrate, an ammonium salt ,and the indicator bromo-thymole blue.

Growth in the medium was shown by turbidity and change in colour of the indicator from light green to blue .due to the alkaline reaction

By using a sterile straight wire, inoculate 3- 4 ml of sterile Kosers citrate medium with a broth culture of the test organism

Incubate the inoculated broth at 35- 37C for up to 4 days, checking daily for growth.

Turbidity and blue color …….positive test citrate utilized (Cheesbrough, 1999).

2-5-3 Coagulase test:-

This test was used to differentiate staphylococcus aureus which produces the enzyme coagulase, from Staphylococcus .epidermdis and Staphylococcus .Saprophyticus which do not produce coagulase

Coagulase causes plasma to clot by converting fibrinogen to fibrin

Two types of coagulase are produced by most strain of Staphylococcus .aureus

Method of slide test (to detect bound coagulase)

Was placed drop physiological saline on each end of slide or two separate slide

Mixed of colony of the test organism in each of the drops to make two thick, suspensions

Plasma was added to one of the suspension and mix gently. Clumping of the organism within 10 second

No plasma added to the second suspension this is use to differentiate any granular appearance of the organism from true coagulase clumping -

Clumping within 10 second …….Staphylococcus aureus

2-5-4 Indole test:-

Testing for indole production is important in the identification of enterobacteria most of strain of E.coli and providence species break down the amino acid tryptophan with the release of indole

The test organism was culture in medium which contain tryptophan .indole production is detected by Kovac,s or Ehrlich,s reagent which contain 4 (p) – dimethylaminobenzaldehyde .this react with the indole to produce a red coloured compound.

Placed an indole paper strip in the neck of the MIU tube above the medium, and stopper the tube .incubated 35-37 C overnight.

Examined for indole production by looking for the reddening of the lower part of the strip

Reddening of strip……positive test indole produced (Cheesbrough,1999)

2-5-5 –Oxidase test:-

The oxidase test used to assist in the `identification of bacteria which produce oxidase enzymes

Apices of filter paper is soaked with a few drops of oxidase reagent .A colony of the test organism was then spread on the filter paper .If the organism is oxidase producing the phenylenediamine in the reagent will be oxidized to a deep purple colour

The method that placed apiece of filter paper in clean petri dish and added 2or 3 drops of freshly prepared oxidase reagent

Then using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and spread it on the filter paper.

Blue purple colour within a few seconds

Result:-

Blue –purple colour(within 10 seconds) ……..positive test oxidase produced (Cheesbrough, 1999)

2-5-6 Oxidation fermentation test:-

This test is used to differentiate those organism that oxidize carbohydrates (aerobic utilization) such as Pseudomonas from those organism that ferment carbohydrate (anaerobic utilization) such as members of enter bacteria.

Principle:-

The test organism was inoculated into two tubes of tryptone or peptone agar medium containing glucose (or other carbohydrate) and the indicator bromothymol blue .the inoculated medium in one tube is sealed with of a layer of liquid paraffin to exclude oxygen

Fermentative organism utilize the carbohydrate in both the open and sealed tubes by a change of colour of the medium from green to yellow oxidative organism however ,are able to use carbohydrate only in the open tube .there is no carbohydrate utilization in the sealed tube(medium remains green)

Although most genera of aerobic bacteria are either carbohydrate utilizers or fermenters, the production of acid may be slow and therefore cultures are usually incubated for 7-14 days

Method:-

Using of sterile straight wire inoculate the test organism to the bottom of two tube of sterile O-F medium .use a heavy inoculums

The inoculated medium in one of the tubes was covered (or one from each carbohydrate pair) with a10 mm deep layer of sterile paraffin oil or molton wax

- Then the tubes were incubated the at 35-37C for up to 14 days examine daily for carbohydrate utilization as shown by acid production.(Cheesbrough, 1999)

Result:-

Open tube	Sealed tube	Interpretation
Yellow	green	Oxidative organism
Yellow	yellow	Fermentative organism
Green blue	green	No utilization of carbohydrate

2-5-7 Urease test:-

Testing for urease enzyme is important in differentiating enterobacteria .Proteus strain are strong urease production..

Principle:-

The test organism is culture in medium which contain urea and be indicator phenol red if the strain is ureas-producing ,the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide .with the release of ammonia ,the medium become alkaline by a change in colour of the indicator to red pink

Red-pink medium …….positive test urease production (Cheesbrough, 1999)

2-5-8 Gram stain:-

Using a sterile wire loop apart of isolates colonies for primary plots and pure were taken and spread on microscopes slides to make thin smears, they were fixed with heat and placed in staining rack .They were covered by crystal violet for two minutes and washed off by tap water, then decolorized with acetone for few seconds and washed off by tap water.

then covered with carbol fuchsine for thirty seconds finally ,the stained smears were washed and air dried .hen they were examined under oil immersion lens (100).The gram positive and gram negative organism shape and arrangement of organism were identified according to (Barrow and feltham ,1993)

2-6 Sterilization:-

2-6-1 Hot air oven

This method was used for sterilization of clean glass containers which were wrapped in paper or out in stainless steel cans ,and the temperature was 160º c (Stainer et al,1986).

2- 6-2-Sterilization by red heat:

the method was used for sterilization wire loops .straight wire and tissue forceps it was done by holding the object over the flame as near and vertical as possible until becomes red –hot (Cruicksshank et al ,1975)

2-6-3-Sterilization by autoclaving:-

This method was used for sterilization of culture media and for material that could not with stand the dry heat .The temperature was 115-112ºc under 10-15 pound pressure for 15-20 minutes (Barrow and feltham ,1993)

2-7 Analysis of data:-

The data of isolated bacteria and questionnaire were used in this study were analyzed by the computer program SPSS

CHAPTER THREE
RESULTS

3-1 Data Collected by samples

3.1.1 Contamination of the farms by E. coli at beginning of poultry production period:

As seen in table 3-1 E. coli revealed the highest contamination level in floor point was (14.3%),then in feeder and feed point were(8.6%) then in drinker was(5.8%) and water was (5.7%),while contamination level of E coli for worker and wall were(2.9%). There was no significantly different ($p \geq 0.05$) contamination level by E coli among the points for begin of Poultry farm production stage.

Table3-1 Percentage of E. coli among the points at beginning of poultry farm production period:

Point	Negative	Positive	Total	Sig
worker	11.4%	2.9%	14.3%	0.08
feeder	5.7%	8.6%	14.3%	
wall	11.4%	2.9%	14.3%	
water	8.6%	5.7%	14.3%	
drinker	8.6%	5.8%	14.3%	
floor	.0%	14.3%	14.3%	
feed	5.7%	8.6%	14.3%	
total	51.4%	48.6%	100.0%	

3.1.2 Contamination of the farms by E. coli at middle of poultry production period:

Table 3-2 E. coli revealed the highest contamination level in worker , water , drinker , floor and feed points were (14.3%) ,then in feeder and wall points were (11.5%). There was no significantly different ($p \geq 0.05$) contamination level of E coli among the points in middle of Poultry farm production period.

Table 3-2 Percentage of E.coli among the points at middle of Poultry production period:

Point	Negative	Positive	Total	Significant Level
worker	.0%	14.3%	14.3%	0.13
feeder	2.9%	11.5%	14.3%	
wall	2.9%	11.5%	14.3%	
water	.0%	14.3	14.3%	
drinker	.0%	14.3%	14.3%	
floor	.0%	14.3%	14.3%	
feed	.0%	14.3%	14.3%	
total	5.7%	94.3.0%	100.0%	

3.1.3 Contamination of the farms by E. coli at end of poultry production period:

As shown in table 3-3 E .coli revealed the highest contamination level in feeder was(11.5%) then in feed and water points were(8.6%),while contamination level of E Coli for drinker and floor were(5.7%).No contamination by E.coli in points of worker and wall. There was no significantly different ($p \geq 0.05$) contamination level by E.coli among the points for end of Poultry farm production period.

Table 3-3Percentage of E. coli among the points at the end of Poultry production period:

Point	Negative	Positive	Total	Sig
worker	14.3%	.0%	14.3%	0.90
feeder	2.9%	11.5%	14.3%	
wall	14.3%	.0%	14.3%	
water	5.7%	8.6%	14.3%	
drinker	8.6%	5.7%	14.3%	
floor	8.6%	5.7%	14.3%	
feed	5.7%	8.6%	14.3%	
total	60.0%	40.0%	100.0%	

3.1.4 Contamination of the farms by Staphylococcus sp at beginning of poultry production period:

As seen in table 3-4 there was no Staphylococcus sp contamination in wall (0 %) .Staphylococcus revealed the highest contamination level in feeder point was (8.6%) ,then in worker , water and floor points were(5.7%) , then in drinker and feed were(2.9%).There was no significantly different ($p \geq 0.05$) contamination level of Staphylococcus Sp the points for begin of Poultry farm production period.

Table 3-4Percentage of Staphylococcus sp among the points at beginning of Poultry production period:

Point	Negative	Positive	Total	Sig
worker	8.6%	5.7%	14.3%	0.53
feeder	5.7%	8.6%	14.3%	
wall	14.3%	.0%	14.3%	
water	8.6%	5.7%	14.3%	
drinker	11.4%	2.9%	14.3%	
floor	8.6%	5.7%	14.3%	
feed	11.4%	2.9%	14.3%	
total	68.6%	31.4%	100.0%	

3.1.5 Contamination of the farms by Staphylococcus sp at middle of poultry production period:

As shown in table Staphylococcus revealed the highest contamination level in wall was (11.4 %) then in feeder point were(8.6%), then in water and floor was(5.7%) ,while contamination level of worker and drinker was (2.9%) and no contamination of staphylococcus in feed. There was no significantly different ($p \geq 0.05$) contamination level of staphylococcus Sp. among the points for end of Poultry farm production period.

Table 3-5 Percentage of Staphylococcus sp. among the points at the middle of Poultry production period:

Point	Negative	Positive	Total	Sig
worker	11.4%	2.9%	14.3%	0.30
feeder	5.7%	8.6%	14.3%	
wall	2.9%	11.4%	14.3%	
water	8.6%	5.7%	14.3%	
drinker	11.4%	2.9%	14.3%	
floor	8.6%	5.7%	14.3%	
feed	14.3%	.0%	14.3%	
total	62.9%	20.0%	100.0%	

3.1.6 Contamination of the farms by Staphylococcus sp at end of poultry production period:

Table 3-6 staphylococcus revealed the highest contamination level in wall , drinker and floor points were(8.6%) ,then in feeder and feed points were (5.8%) and no contamination of staphylococcus sp in worker point There was no significantly different ($p \geq 0.05$) contamination level of staphylococcus sp among the points at the mid of Poultry farms production period.

Table 3-6- Percentage of Staphylococcus sp among the points at the end of Poultry production period:

Point	Negative	Positive	Total	Sig
worker	8.6%	.0%	14.3%	0.87
feeder	5.7%	5.7%	14.3%	
wall	5.7%	8.6%	14.3%	
water	8.6%	5.7%	14.3%	
drinker	5.7%	8.6%	14.3%	
floor	5.7%	8.6%	14.3%	
feed	8.6%	5.8	14.3%	
total	48.6%	51.4%	100.0%	

3.1.7 Contamination of the farms by Salmonella sp at beginning of poultry production period:

Table 3-7 Salmonella Sp revealed the highest contamination level in floor point was(11.5%), then in feed, drinker and wall points were (11.4%) ,then in feeder and water points were (8.6%) ,then in worker point was(5.8%). There was no significantly different ($p \geq 0.05$) contamination level of salmonella among the points at the begin of Poultry farm production period.

Table 3-7-Percentage of Salmonella sp among the points at the beginning of Poultry production period :

Point	Negative	Positive	Total	Sig
worker	8.6%	5.8%	14.3%	0.57
feeder	2.9%	8.6%	14.3%	
wall	2.9%	11.4%	14.3%	
water	5.7%	8.6%	14.3%	
drinker	2.9%	11.4%	14.3%	
floor	2.9%	11.5%	14.3%	
feed	5.7%	11.4%	14.3%	
total	31.4%	68.6%	100.0%	

3.1. 8 Contamination of the farms by Salmonella sp at middle of poultry production period:

As seen in table 3-8 Salmonella sp in feed the highest contaminated point (14.3%) , then floor was(11,4%) and wall, water were(8.6%),then in worker, feeder and drinker points were(5.7%). There was no significantly different ($p \geq 0.05$) contamination level of salmonella among the points at the end of Poultry farm production period.

Table 3-8 Percentage of Salmonella sp among the points at the middle of Poultry production period:

Point	Negative	Positive	Total	Sig
worker	8.6%	5.7%	14.3%	0.04
feeder	8.6%	5.7%	14.3%	
wall	5.7%	8.6%	14.3%	
water	5.7%	8.6%	14.3%	
drinker	2.9%	5.7%	14.3%	
floor	2.9%	11.4%	14.3%	
feed	.0%	14.3%	14.3%	
total	34.3%	65.7%	100.0%	

3.1.9 Contamination of the farms by Salmonella sp at end of poultry production period:

As shown in table 3-9 Salmonella Sp revealed the highest contamination level in drinker was (14.3%) then in feed point was(11.5%) then in water was (5.7%) ,while contamination level of Salmonella Sp in worker, feeder and floor were (2.9%). There was no significantly different ($p \geq 0.05$) contamination level of Salmonella Sp among the points at the end of Poultry farms production period.

Table 3-9 Percentage of Salmonella Sp among the points at the end of Poultry production period:

Point	Negative	Positive	Total	Sig
worker	11.4%	2.9%	14.3%	0.00
feeder	11.4%	2.9%	14.3%	
wall	14.3%	.0%	.3%	
water	8.6%	5.7%	14.3%	
drinker	.0%	14.3%	14.3%	
floor	2.9%	2.9%	14.3%	
feed	2.9%	11.5%	14.3%	
total	51.4%	48.6%	100.0%	

3-2 Data collect by questionnaire:

3-2-1 Fences of farms, nearest farm and distance between the different ages of poultry, control of transferring of the equipment and tools between farms:

As shown in table 3-10- (46.7%) of the semi close farms were found fenced, while (43.3%) of the open farms were found fenced and 10% of it were not fenced. There was no significantly different ($p \geq 0.05$) to found to fence between Semi close and open Poultry farm in this survey

The recorded data of the questionnaire indicated that there was no significantly different ($p \geq 0.05$) nearest farm between Semi close and open Poultry farm in this case. The 2 km distant of nearest farm between semi close and open Poultry farm were(3.3%) and 6.7% ,and 5k m were(6.7%), (10.0%) respectively and less than 2km were(36.7%) for both farms.

As seen in table 3-10 200 m the distance between the different poultry ages were(13.3%)semi close farm and(6.7%) in open farm . Less than 200 m the distance between the different ages were(33.4%) in semi close farm and(46.6 %) in open farm there was no significantly different ($p \geq 0.05$) of the distance between the different ages between semi close and open Poultry farm in Khartoum state.

The present results showed that farms were controlled transfer the equipment and tools in semi close farm (41.4%) and in open Poultry farms (30.0%). The farms were not found to be control were (3.4%) and (24.1%) in Semi close and open Poultry farms.

Table 3-10 fences, nearest farm and distance between different ages and control of equipment and tool between farms:

	Measurement			
Did the farm fenced in all direction:	Semi close	Open	Total	Significant Level
Yes	46.7%	43.3	90%	0.93
No	0.0%	10.0%	10%	
How far is the nearest farm:				
5 km	6.7	10.0	16.7	
2 km	3.3	6.7	10.0	
Less	36.7	36.7	73.3	
What are the distance between the different ages				
20	13.3	6.7	20.0	0.19
Less	33.4	46.6	80.0	
Are there control for transfer of equipment and tools between farms				
Yes	41.4	31.0	72.4	
No	3.4	24.1	27.6	

3.2.2 The ways of prevention entering of other animals and wild birds and disinfection measure:

Table 3-11 presented the ways to prevent the entry of other animal (cat, dog, rat and wild birds) in semi close and open Poultry farm in this study. The study explained that the most of owners in semi close farms (40.0%) and (6.7%) were haven't ways. Also data shown that (16.7 %) of owners in open farm have ways to prevent the entry of other animal. The results indicated high significant differences ($P<0.001$) for prevention entry of (cat, dog, rat and wild birds) in semi close and open Poultry farm in this survey.

The spray disinfectant for cars at the entrance and the vehicle in the bathing were found in Table (3.11). The study showed that all owners in open farm (0.0%) and a few in semi close farm (13.3%) did not used spray disinfectant for cars at the entrance .While 40 % of semi close farm and (10%) of open farm used the vehicle in the bathing. There was insignificant association between spray disinfectant for cars at the entrance and the vehicle in the bathing in semi close and open Poultry farm.

Table3-11 the ways to prevention the entry of other animal (cat, dog, rat and wild birds) and disinfection:

	Measurement			
Are there any ways to prevent the entry of other animal (cat ,dog and rat)	Semi close %	Open %	Total %	Significant Level
Yes	40.0	16.7	56.7	0.00
No	6.7	36.7	43.3	
Are there any method of protection to prevent the entry of wild birds				
Yes	40.0	16.7	56.7	0.00
No	6.7	36.7	43.3	
Is there spray disinfectant for cars at the entrance				0.02
Yes	13.3	0.0	13.3	
No	33.3	53.4	86.7	
Did the vehicle in the bathing				
Yes	40.0	10.0	50.0	0.00
No	6.7	43.3	50.0	

3.2.3 Rules for entering visitors into poultry farms :

Table 3-12 presented the frequencies of farm owners having rules for enter visitors into these farms. The data showed that most of owners in open farm (43.4%) have no rules for enter visitors into their farms. On other hand 23.3%of them in Semi close farm they have rules for enter visitors into their farms. There was no significantly different ($p \geq 0.05$) between these two system farms

The showing, protective clothing, Protective boot, disinfectant hands when entering, and other as rules to enter visitors in close and open Poultry farm in study were(43.3%) ,(0.0%), (0.0%) ,(0.0%) and(20.0%) in semi close were (26.7%),(0.0%),(0.0%) ,(0.0%) and (3.3%) in open .There was no significantly different ($p \geq 0.05$) between Showery, protective clothing, protective boot, disinfectant hands when entering and other rules to entering visitors in Semi close and open Poultry farm in this survey .

Table 3-12 Rules for enter visitor in to the farm (showering protective clothing protective boot, disinfectant hand):

	Measurement			
Are there a rule to enter visitors	Semi close	Open	Total	Significant Level
Yes	23.3	10.0	33.3	0.08
No	23.3	43.4	66.7	
What are rules to enter visitors				
Showering	43.3	26.7	70.2	
Protective clothing	0.0	0.0	0.0	
Protective boot	6.7	0.0	6.7	
Disinfectant hands when entering	0.0	0.0	0.0	
More than one rule	20.0	3.3	23.3	

3.2.4 Sampling taken of feed ingredients before and after industries:

Table 3-13- The results indicated that sampling taken of feed available about(20.0%) in semi close) and(3.3%) in open of farms under investigation indicated that there significantly different ($p \geq 0.05$) between two system .The result shown that there no processing in case found pathogens in open farm (0.0%) and few in close farm (33.3%)

The collection of data was shown that only (16.7%) in Semi close and (6.7%) in open. Poultry farm take samples of water, and time period for taking samples of water by year. 6 month and more were (52.4%) (, 14.3%) and (0.0%) in semi close farm and (23.8%), (4.8%) and (4.8%) in open farm.

Table 3-13sampling taken from feed ingredient before and after industrialization processing if found pathogen and take sample of water and period time:

	Measurement			
Are there sampling taken of feed ingredients before industrialization	Semi close %	Open %	Total %	Significant Level
Yes	20.0	3.3	23.3	0.02
No	26.7	50.0	76.7	
Are there sampling taken of feed ingredients after industrialization				
Yes	20.0	3.3	23.3	0.02
No	26.7	50.0	76.7	
Are there any processing in case found pathogens				0.05
Yes	13.3	0.0	13.3	
No	33.3	53.4	86.7	
Do they take samples of water				
Yes	16.7	6.7	23.3	0.14
No	30.0	46.7	76.7	
What the time period for take samples of water				
Year	52.4	23.8	76.2	
6 month	14.3	4.8	19.0	
More	0.0	4.8	4.8	

3.2.5 Samples from houses, and time period for taken samples:

As seen in table 3-14 a few of farm owner in semi close (6.7%) and in open farm (3.3%) taken samples to measure the effectiveness of the process after cleaning and disinfecting farm.

The present results showed that only 3.3% in both close and open farm of owner farms were found to reviewing the process for problem If found the cause of disease. The data also showed that there were no processing in case found pathogens (0.0%) in open farm and (13.3%) in semi close farm.

To get rid the dead birds' used in table 3-14

Table 3-14sampling taken of house after cleaning and disinfectant, processing in case found pathogen agent and how to disposal dead birds :

	Measurement			
After cleaning and disinfecting farm are samples are taken to measure the effectiveness of the process	Semi close	Open	Total	Significant Level
Yes	6.7	3.3	10.0	0.48
No	40.0	50.0	90.0	
If you found the cause of disease reviewing the process for problem				
Yes	3.3	3.3	6.7	0.92
No	43.3	50.0	93.3	
Are there any processing in case found pathogens				
Yes	13.3	0.0	13.3	
No	33.3	53.4	86.7	
How to get rid the dead birds disposal				0.03
Out door	0.0	10.0	10.0	
Incinerator according to the specification	26.7	3.3	30.0	
Burning in hole	20.0	40.0	60.0	

3.2.6 Check health, protection of worker when entering the farm and mixing between worker:

Table 3-15 results showed that most of farms were checked health periodically for worker (6.7%) in semi close system and (3.3%) in open farms. The Showery, Protective clothing, Protective boot, disinfectant hands when entering and other procedures for worker when entering the farm were (0.0%),(0.0%), (23.3%) (0.0%) and (23.3%) in semi close farms. (0.0%), (3.3%), (13.3%)(,6.7%) and (30.3%) in open farms. Mixing between worker (30.0%) in semi close system and (16.7%) in open system.

Table3-15 worker check health, procedure for worker when entering the house and mixing between workers:

	Measurement			
Are health check periodically for worker	Semi close	Open	Total	Significant Level
Yes	6.7	3.3	10.0	0.48
No	40.0	50.0	90.0	
What re the procedures for worker when entering the farm				
Showery	0.0	0.0	0.0	0.63
Protective clothing	0.0	3.3	3.3	
Protective boot	23.3	13.3	36.7	
Disinfectant hands when entering	6.7	0.0	6.7	
Others	23.3	30.3	53.3	
Is any mixing between worker from different farm	33.3	53.4	86.7	0.07
Yes	30.0	16.7	46.7	
No	16.7	36.7	53.3	

CHAPTER FOUR
DISCUTION

The environment of broilers houses in modern poultry production system is usually contaminated with a huge number of different microbial components

E. coli Found throughout the world, these organisms can infect every wide variety of hosts including invertebrate and vertebrate wildlife, domestic animals, and humans. The contamination by E.coli in the beginning revealed the highest contamination level in floor point, then in feeder and feed point then in drinker and water while contamination level of E. coli for worker and wall were lowest

The contamination by E coli in the mid revealed the highest contamination level in worker, water, drinker, floor and feed points then in feeder and wall .The contamination by E coli in the end revealed the highest contamination level in feeder then in feed and water points while contamination level of E. coli for drinker and floor .Infection by E coli in different points due to the biosecurity measures level this agree with (McGruder et al,1998)The risk for coli bacillosis increases with increasing infection pressure in the environment. A good housing hygiene and avoiding overcrowding are very important. Other principal risk factors are the duration of exposure, virulence of the strain, breed, and immune status of the bird.

Staphylococcus sp infections are common in poultry. Staphylococcus sp revealed the highest contamination level in feeder point the beginning .Staphylococcus revealed the highest contamination level in wall point while contamination level of worker and drinker were lower and no contamination of staphylococcus in feed in middle .Staphylococcus revealed the highest contamination level in wall , drinker and floor points and no contamination of Staphylococcus sp in worker in the end

of period , infection Staphylococcus sp due to condition of bad management farmer and not applied the hygienic practice in infections farms this agree with (Awan and Matsumoto, 1997) found coagulase-negative staphylococci were isolated from commercial broiler chickens from different points.

Salmonella sp revealed the highest contamination level in floor point then in feed , drinker and wall points, then in feeder and, then in worker point in the beginning . In the middle of production period Salmonella in feed was highest contaminated point,. In the end of production period Salmonella sp revealed the highest contamination level in drinker then in feed point then in water, while contamination level of Salmonella sp in worker, feeder and floor were low. (Rose *et al*.1999) reported that like many other bacterial, salmonella can be transmitted in several ways, such as worker, feeder, wall, water, drinker, floor and feed. Feeds have often been identified as likely sources of *Salmonella* because of contaminated animal proteins and other ingredients. Feed is another potential source of introduction of salmonellae into finished feeds (Jones *et al*,1999). studies have demonstrated that chicks can be readily infected by very low levels of salmonellae in their feed. The contamination by salmonella in the begin of rearing period in feed was 11.4% this in same line with (C.Marin et al,2010) that feed contamination in 17% of cases by Salmonella sp that due to rodents that could acquire the infection from inaccessible parts of the house may deposit contaminated dropping directly into feeding system (Davies and Breslin,2003) . 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. At the end of rearing period the spread of Salmonella was confirmed,(48.6%) of the houses study were contaminated similar result demonstrating a Salmonella spreading during growing and increase of the prevalence in poultry houses at the end of growing period have

been reported by the other (Irwin et al., 1994;Wray and Davies, 1994; Davies and Waray, 1996).

The results of questionnaire for evaluate biosecurity status in poultry farm in Khartoum state showed that 46.7% of the close farms were found fenced, while 43.3% of the open farms were found have fence, this fence could be used to stop people and animals of entering the farm as control diseases, this result in same line with (Mahmoud et al., 2014) who reported that majority of the poultry farms in Khartoum states (77.8%) had a secure boundary fence

The recorded data of the questionnaire indicated that there was no significantly different ($p \geq 0.05$) nearest farm between semi close and open Poultry farm in this case. Distance of nearest farm between semi close and open system, 5km were 6.7%, 10.0% respectively, this study showed that the most farm didn't apply the ideal condition of the distance between farms, leading to spared the disease and didn't control the infection between farms this result in same line (Mahmoud et al 2014).

200 m the distance between the different ages were13.3%semi close farm and 6.7% in open farm there was no significantly different ($p \geq 0.05$) the distance between the different ages between Semi close and open Poultry farm in Khartoum state, this result agree with (Mahmoud et al ,2014).

The control of the equipment and tools in semi close farm (41.4%) and in open Poultry farms (30.0%)., this results showed that higher careful attention in semi close than open one this due to owner of semi close have few attention to transfer their equipment and tools than owner of open system this result was similar with they reported that(Mahmoud et al ,2014). Farmers practice of bio-security in this study showed that farms have no a good understanding of the entire of cat, dog, rat and wild birds. The study explained that the most of owners in close farm (40.0%) not have ways to prevent the entry of cat ,dog, rat and wild birds and lowest

proportion of them (6.7%) were not have ways. Also data shown that 16.7 % of owners in open farm have ways to prevent the entry of this animal. The results indicated high significant differences (P<0.001) presented the ways to prevent the entry of other animal (cat ,dog, rat and wild birds) in semi close and open Poultry farm in this survey. This study showed that farmer's owners who understand the purposes of a bio-security measure are more likely to have rules for enter visitors into their farms. The data showed that most of owners (66.7%) in open and semi farm have no rules for enter visitors into their farms. This was in agreement with (Mahmoud et al, 2014).who reported that about 88.9% of the farms have no rules for enter visitors into their farms

Dead birds to rendering facilities off farm as having the highest association with infected diseases .Outdoor, Incinerator according to the specification, a burning in hole methods to get rid the dead birds disposal were 0.0%, 26.7% and 20.0% in close farm and 10.0. .3% and 40.0% in open farm this study was in agreement with the study of (Vieira et al,.2009) who found that on-farm bird disposal was by 100% of the producers in their study area. Results from a similar study (Ali et al., 2014) showed that 6.2% of farms in the open system in Khartoum state left dead birds thrown away. (Dorea et al.2010) reported that the practice of disposing birds' off-farm may pose a higher risk of pathogen spread.

The results indicated that sampling taken of feed ingredients before and after industrialization poultry farms were found to be available 20.0% and 3.3% in semi close and open of farms under investigation indicated that there significantly different ($p \geq 0.05$) this agree with(Mc Ellhiney ,1981) .

The result shown that there no processing in case found pathogens in open farm (0.0%) and few in close farm (33.3)

The result shown that only (16.7%) and (6.7%) Semi close and open Poultry farm which data collect from it take samples of water this agree with (Amy E.

Halls,2008) .The present results showed that most of farms were found to be check health periodically for worker were 6.7% and 3.3% in Semi close and open farm this due to worker are not stay in farm for long time. The showery, protective clothing, protective boot, disinfectant hands when entering and other procedures for worker when entering the farm were 0.0, 0.0, 23.30.0 and 23.3 in semi close farm and were 0.0, 3.3, 13.3,6.7 and 30.3 in open farm. 30.0% and 16.7 % mixing between workers from that because they don't know the idea of biosecurity.

Conclusion

The biosecurity measures level among poultry farms in Khartoum state could be classified into low in both open and semi close system and application of HACCP system in farms were not possible now.

Salmonella Sp, E.coli and StaphylococcusSp could be introduced into poultry flocks from many different sources. Such as worker, feeder, wall, water, drinker, floor and feed

Recommendations

- Government policy needs to facilitate the improvement of biosecurity adoption among poultry farmers.
- Routing visiting from veterinary authorities, and applied the law that every farm must have veterinary supervision.
- Extension diseases prevention measures services among poultry owners farmers and labors.
- Encouragement of annual fairs and workshops of HACCP which deal with the poultry sector in Khartoum State.
- Advice training for farmer owners and staff who work in poultry beside hygiene must be improved and updated this program.

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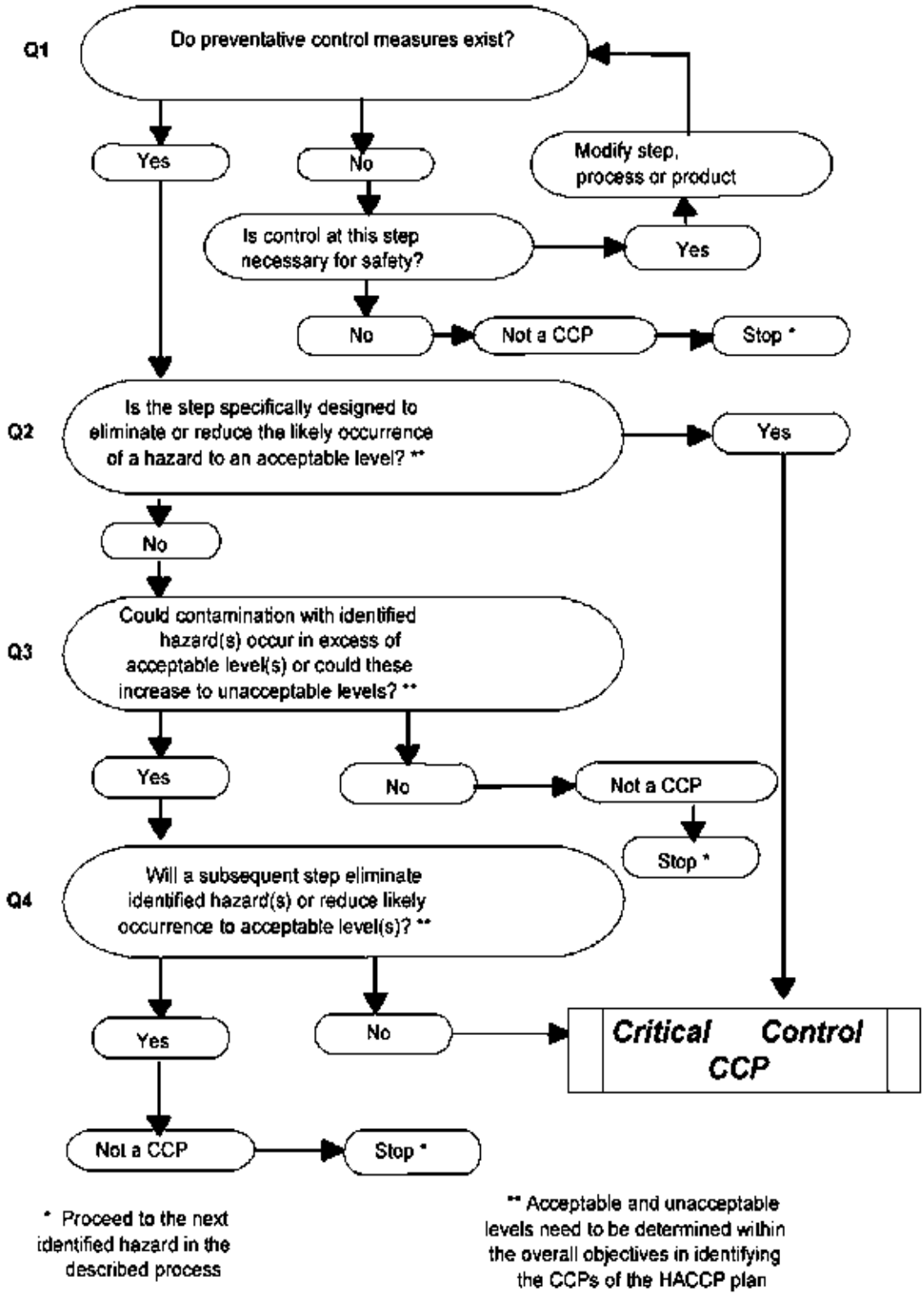
Plate (1)Media XLD Salmonella

Plate(2)Media : Manitol salt agar staphyllococcus

Plate(3)Media:EMB E coli

DIAGRAM (1). EXAMPLE OF DECISION TREE TO IDENTIFY CCP$_S$ (answer questions in sequence)



Sudan University of science and technology

Faculty of Graduate Studies

Questionnaire of Master Research

About Application of HACCP in Biosecurity in Poultry Farms

1-did the farm fenced in all direction?

A-yes ( ) b-no ( )

2-how far is the nearest farm?

A-5000km

b- 2000 km

c- Less

3- Are there any ways to prevent the entry of other animal (cat dog .rat)

A-yes ( ) b-no ( )

4- are there any method of protection to prevent the entry of wild birds ?

Yes ( ) no ( )

5- what are the distance between the different ages ?

A-20 ( ) B-less ( )

6-are there a rules to enter visitors?

a-yes ( ) B- no( )

7-if are found what are that?

A-showering ( )

B-protective clothing ( )

c-protective boot ( )

D-disinfectant hands when entering ( )

8-is there spray disinfectant for cars at the entrance ?

A-yes ( ) b- no ( )

9- Did the vehicle in the bathing ?

Yes ( ) B-no ( )

10- Are there sampling taken of feed ingredients before industrialization ?

Yes ( ) no ( )

11- are there sampling taken of feed ingredients after industrializationare there any processing in case of found pathogens ?

Yes ( ) no ( )

12- are there any processing in case of found pathogens ?

Yes ( ) no ( )

13- do they take samples of water ?

Yes ( ) no ( )

14- what the time period ?

-year ( ) 6 month ( ) more ( )

15- after cleaning and disinfecting farm are samples are taken to measure the effectiveness of the process ?

Yes ( ) no ( )

16- if you found the cause of disease reviewing the process for problems?

Yes ( ) no ( )

17-are health check periodically for worker?

A yes ( ) b- no ( )

18- What are the procedures for worker when entering the farm :

Showering ( )

Protective clothing ( )

Dipping boot in disinfectant ( )

-hand disinfectant ( )

19- is any mixing between worker from different farm ?

Yes ( ) no ( )

20-how to get rid the dead birds disposal ?

Out :

A- out door

B- Burring

C- Burning in hole

D- Incinerator according to the specification

21- Are there control for transfer of equipment and tools between farms?

Yes ( ) no ( )