Sudan University of Science and Technology

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Identification of the Food Contaminating Pathogenic Bacteria and Their Load in the CCPs of Canine Feeding Process, in AlAin Police K9, (Abu Dhabi Emirate)

تحديد البكتيريا المسببة للأمراض الملوثة للأغذية وأحمالها في نقاط التحكم الحرجة في عملية تغذية الكلاب البوليسية العين (ابو ظبي- الامارات)

By

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Dedication

To the soul of my father and mother.

To my wife,

To the candles of my live, my children, from whom I drive the power to carry out this work.

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List of Abbreviations

K9	Homophone of "canine". Referred to Police Dogs
CFU	Colony Forming Unit
TVC	Total Viable Count
MRSA	Methicillin-Resistant *Staphylococcus aureus*
HACCP	Hazard Analysis and Critical Control Points
CCPs	Critical Control Points
FAO	Food and Agriculture Organization
WHO	World Health Organization
FDA	Food and Drug Administration
FEDIAF	European Pet Food Industry Federation
CDC	Centre for Disease Control
AIFS	Australian Institute of Food Safety
APHA	Animal and Plant Health Agency
NASA	National Aeronautics and Space Administration

Abstract

This study was conducted in Al-Ain (Abu Dhabi Emirate) at the Police Dogs Unit K9, during period from December, 2018 to January 2019 to detect the load of bacterial contamination at the critical control points of canine feeding process and these were kennel floor, handlers' hands, the bowls before and after meal and meat canned food. A number of 300 swabs samples were taken from the five points on duration of ten consecutive weeks. The samples were sent to the Central Veterinary Laboratory for Microbiological analysis. Where the results displayed a variety of contaminants were identified at the stages of the feeding processes, a highest bacterial viable counts were at the kennel floor (4.21 $\log_{10}$cfu/cm^2) and the Bowls after meal (5.05 $\log_{10}$cfu/cm^2), while the low or nearly neglected bacterial count was in the canned food (0.78 $\log_{10}$cfu/cm^2). *Staphylococcus aureus* and *Escherichia coli* were isolated at lower mean bacterial count at points of handlers' hands (0.34%, 0.06%) and canned food (0.00%, 0.06%), while they were highest at the kennel floor (25.54%, 60.05%) and the Bowls after meal (70.89%, 36.46%), respectively. This study has shown that the highest bacterial contamination in Police dogs' feeding processes is at the bowls after meal and kennel floor and the lowest at the handler's hands and the canned food, and also that *Staphylococcus aureus* and *Escherichia coli* were the major pathogenic contaminants in the feeding processes.

الملخص

تم اجراء هده الدراسة بوحدة الكلاب البوليسية بمدينة العين بإمارة ابوظبي لمعرفة حجم ونوع التلوث البكتيري في نقاط التحكم الحرجة عند تغدية الكلاب. وهي أرضية الحظيرة، وأيادي العمال، صحون الاكل قبل وبعد الوجبة، واللحم المعلب.تم اخذ 300 عينه (مسحة) من هده النقاط على مدي 10 اسابيع. تم ارسال العينات الي المختبر البيطري المركزي للتحليل ومعرفة حجم التلوث وانواع البكتريا المسببة له. اظهرت التحاليل اعداد البكتريا ونوعين من البكتريا المسببة للتلوث وهي: *المكورات العنقودية الذهبية والإشريكية القولوني* أظهرت النتائج أنه تم التعرف على مجموعة متنوعة من الملوثات في مراحل عملية التغذية، وكان أعلى عدد من التلوث البكتيري في أرضية الحظائر (4.21) وصحون الأكل بعد الوجبة (5.05)، في حين أن حجم التلوث البكتيري كان أكثر انخفاضا في اللحم المعلب (0.78). تبعا لذلك، كانت المكورات العنقودية الذهبية *والإشريكية القولونية* في عدد بكتيري أقل في أيادي العمال (0.06% ,0.34%)واللحم المعلب (0.00% ,0.06%)، بينما كانت أعدادها أعلى في أرضية الحظائر ((60.05% ,25.54% وصحون الأكل بعد الوجبة، على التوالي. أظهرت هذه الدراسة أن أعلى تلوث بكتيري في عمليات تغذية الكلاب البوليسية يكون في صحون الأكل بعد الوجبة وأرضية الحظائر بينما يكون الأدنى في أيادي العمال واللحم المعلب، وكذلك أن المكورات العنقودية الذهبية والإشريكية القولونية كانت الملوثات المسببة للأمراض الرئيسية في عمليات التغذية.

Introduction

The Hazard Analysis and Critical Control Points (HACCP) system has become a synonymous with food safety. It is a worldwide-recognized systematic and preventive approach that addresses biological, physical and chemical hazards through anticipation and prevention rather than through end-product inspection and testing (FAO, 1998).

The HACCP concept was pioneered in the 1960s by the Pillsbury Company, the United States Army and the United States National Aeronautics and Space Administration (NASA) as a collaborative development for the production of safe foods for the United States Space Programs. NASA wanted a Zero defects program to guarantee the safety of foods that astronauts would consume in space. Pillsubry, therefore, introduced and adopted HACCP as the system that could provide the greatest safety while reducing dependence on end-product inspection and testing. HACCP emphasized control of the process as far upstream in the processing system as possible by utilizing operator control and/or continuous monitoring techniques at critical control points. Pillsbury presented the HACCP concept publicly at conference for food protection in 1971. The use of HACCP principle in the promulgation of regulations for low-acid canned food was completed in 1974 by the United States Food and Drug Administration (FDA). In the early 1980s, the HACCP approach was adopted by other major food companies (FAO, 1998).

Canning process aims to prevent food spoilage and preserve the quality of the food, so that the food can be kept for an extended period of time without refrigeration and without loss of nutrition values (Blumenthal, 1990). Commercial molds, these microorganisms can be acquired during handling and processing, surviving any preservation treatment, and contaminating food in storage.

The principal pathogenic microorganisms that had been documented associated with spoilage of human canned food are *Clostridium botulinum*, *Salmonella spp.* and enterotoxin producing *Staphylococcus aureus.* Among them, *Staphylococcus spp.* rank first in terms of frequency, followed by *Clostridium botulinum* and *Salmonella spp.* (Hersom and Hulland, 1980). Microorganisms that contaminate processed pet food are responsible for digestive tract diseases, such as diarrhea, vomiting, nausea and abdominal pain. Improper storage of opened canned food is another factor contributing to spoilage of canned food. Environmental temperature and oxygen availability influence the bacterial growth in opened canned food (FDA, 2019).

Occasionally, the outcome of these diseases on pets is life-threatening and it requires some preventive measures to precede and forbid the eventuality of any food-borne illness. Thus, having the knowledge about the biological culprits, and determining the critical points in the feeding process can help in decreasing the chances of food-borne illness incidences (FDA, 2019).

The police dogs; or K-9 as known internationally, are dogs specifically trained to assist in law enforcement. It plays a significant role in maintaining security across the Emirate of Abu Dhabi. The most common breeds are German shepherd, Belgian Malinois, Labradors and others. Their duties include drugs and explosives detection, locating missing people, finding evidences in crime scenes, riot control, and cadavers' search.

The kennels in the Unit are designed with the standard specification to maintain the health and welfare of the dogs. The kennel floors are made up of concrete tiles cleaned and disinfected twice daily, regular water supply, food is purchased from known wholesome company, feeding bowls are of stainless steel to ensure efficient disinfection, air condition to combat the hot weather of gulf area, and water-

sprinkler fans are installed in the kennel yard. The laborers are well trained to clean and disinfect the kennels and how to offer the food for dogs.

Objectives:

The objectives of this study were:

- To determine the total viable count of bacteria at different critical points of dogs feeding processes.
- To isolate and identify the type of the feed contaminating bacteria.

Chapter One

Literature Review

1.1. Types of Dog Feed

The best food is the one that meets all the nutritional requirements necessary for dog health. There are five main Types of dog food, they are firstly the Kibble or Dry Food which is the most economical type of commercial food. It can be stored for long time without refrigeration. Since it is crunchy, chewing will help to keep the dog's teeth healthy. Secondly, is canned food, and typically dogs prefer canned or wet food. It has long shelf live. Canned food is 75% water. The high-water content indicates less nutrient value. The best brand is the one provides much digestible protein. Thirdly semi-moist food, it is shaped like pork chops, burgers, or other meaty foods. It is of least nutritional value of all dog's food and contains many artificial flavors and colorings. Not considered as diet for dogs. The fourth type is the Home Cooked, which allows the owners to be sure that the nutritional needs of their dogs being met. It is time consuming and expensive. The last type is the Raw Food, it consists of raw meat with some bones and organs mixed in, as bones are natural sources of calcium and phosphorus. This type of food works well for many dogs (Nylabone, 2019).

1.2. Dog Dietary Requirements

A balanced dog diet is critically important to the dog's cell maintenance and growth and overall health. It must contain specific levels of protein, fat, vitamins, and minerals depending on the life stage of the dog. There are six main classes of nutrients for optimal health (AIFS, 2016)

1.2.1 Water

Water is essential to life; it counts 60-70% of adult dog body weight. While food can meet some of the dog needs (dry food 10% of moisture, canned food about 70%), but still fresh water should be available all the time. Serious illness occurs when body water is decreased by 10%, where up to 15% can result in death.

1.2.2 Protein

They are the building blocks for cells, tissues, organs, enzymes, hormones and antibodies which are essential for growth, maintenance, reproduction, and repair. The protein sources like animal-based meat as poultry, beef, turkey, fish, and eggs. Also, in vegetables and cereals.

1.2.3 Fats

Fat is the most concentrated form of food energy; supplies twice the energy produced by proteins and carbohydrates. Essential for cell structure, production of some hormones, helps absorption of some vitamins; A, E, D and K. Efficiency of essential fatty acids (Linoleic acid) results in reduced growth and skin problems.

1.2.4 Carbohydrates

Provides energy, and it is vital for health of intestine and reproduction. Fibers can alter the bacterial population in the small intestine and can help manage chronic diarrhea in dogs. The moderately fermentable fibers (Beet pulp) are used in dog foods to promote healthy gut. Unlike highly fermentable fibers which cause flatulence and excess mucus. Bran of corn, rice, and wheat are examples of moderately fermentable fibers.

1.2.5 Vitamins

Vitamins are necessary for normal metabolic function. When feeding balanced diet, it is advised not to add vitamin supplements, unless deficiency is diagnosed.

1.2.6 Minerals

Minerals are important for bones and teeth health, fluid balance, and some other metabolic processes. (Laverdure-Dunetz, 2018)

1.3 Digestive System

Dogs are evolved to eat bacteria in their food and from other sources, they are naturally coprophagic; eating feces of their own and other animals. There are some factors that contribute in preventing pathogenic bacteria to take foothold in dog body. Saliva is the first defense line or the gate keeper for its protective role against harmful pathogens entering with food. The stomach with its high acid (PH 1-2) Hydrochloric acid (HCL) is the right media for enzymes to work efficiently, and kills bacteria escaped from the action of saliva enzymes (Carnivora, 2019). The pH varies from region to region, with becoming neutral or slightly alkaline towards the distal part of GI tract, but that of the colon is (6.5), turns the fecal PH to 6 or less (Davis *et al* ,1997) due to production of acids by bacterial fermentation in the lower gut. Bifidobacteria are major component of the microbial barrier to the infection, as they produce antimicrobial agents active against Gram-negative and positive organisms (Gibson and Wang, 1994). Due to the high acidic pH of the stomach, only limited number of microbes can thrive, approximately less than $\mathbf{10^4}$ cfu/ml. Towards the small intestine, the digestive enzymes and juices make the PH nearly alkaline, facilitates the growth of many facultative and strictly anaerobic bacteria including Streptococci, Lactobacilli, Enterobacteria, Bifidobacteria, Bacteroides etc. The bacterial population reaches up to $\mathbf{10^8}$ to $\mathbf{10^9}$ cfu/ml of content.

Colon is greatly populated with bacteria $\mathbf{10^{11} - 10^{12}}$ cfu/ml of contents (Cummings, *et al*., 1989). The bacteria that survive the high acid of stomach pass to the small intestine. In the duodenum, the liver and pancreas deposit their enzymes, bicarbonate and bile salts, respectively. The pancreatic enzymes digest the cell walls of harmful bacteria. The bile salts are primarily used for fat digestion and transportation, and also potent antimicrobial agents. Lysozyme is secreted by cells lining of the digestive tract, and it is a potent enzyme that attacks bacterial cell walls and prevents bacterial overgrowth in upper gastrointestinal tract. For bacteria to survive and to stick to the intestinal wall, they must have contained adhering proteins, locomotion, and rapid multiplication to overcome the forward peristaltic movement of small intestine, otherwise they will pass to the fecal matter and into the large intestine. Higher number of bacteria found in the large intestine, mainly gram-negative which are very important to normal large intestine physiology. The normal dog flora is generally affected by the dog diet, environment, and immunity (Pilla and Suchodolski, 2020). The intestinal flora is relatively stable and maintains constant number and type of bacteria in each part of the intestine that will discourage infection and prevents overgrowth of pathogenic bacteria. The growth of foreign microorganisms entering the system with food and water is inhibited by the action of antimicrobial substances produced of the intestinal flora. The resident microbiota of the canine GI tract, plays a major part in the defensive mechanism. The large population of beneficial bacteria prevents pathogen colonization by occupying receptor sites and competing for space and nutrients etc. (Gibson and Wang, 1994). Dogs, for instance, are quite resistant to *Salmonella* and require a large dose to cause infection, however, small dose can cause infection when flora is suppressed by oral antibiotics which upsets the floral balance and lead to pathologic overgrowth. (Carnivora, 2019).

1.3.1 Oral bacterial flora of dogs

In study done on the bacterial flora of the dog oral cavity and of a bite wound, aerobic bacteria were isolated from mouth swabs of 16 normal and 5 rabid dogs as well as from infected dog-bite wounds from 18 patients. A total of 20 species was isolated from mouth swab culture. The most isolated organisms from the dog saliva are *Klebsiella pneumoniae spp pneumoniae, Escherichia coli, Staphylococcus aureus, Citrobacter freundii, Enterobacter cloacae*, *Acinetobacter calcoaceticus* and *Pasteurella* species. There was no difference in the aerobic bacteria flora between rabid and non-rabid dogs. Dewhirst and his colleagues (Dewhirst *et al*., 2012) identified more than 400 kinds of oral bacteria in dogs. Some bacteria in dog saliva can cause severe diseases and death in human like *Capnocytophage canimorsus*, it can be spread to people through a bite or after close contact with dog (Kasempimolporn *et al.*, 2003).

1.4 Food Contamination

When food is subjected to unsafe handling and cooking, the disease-causing organisms like bacteria, viruses, or parasites can contaminate the food. They also produce toxins that leads to food intoxication. Other contaminants like presence of pesticides or cleaning compounds may result in food contamination.

The reasons for contamination are improper handling and preparation, unsound storage system, poor personal hygiene, inadequate cleaning and sanitization of utensils and contamination by flies and other pests (Argudin *et al.*, 2010)

1.4.1 Types of Food Contamination

They are four categories; Biological, chemical, physical and cross contamination.

1.4.1.1 Biological contamination

It is the contamination of food by other living organisms, it is the most common type of poisoning and food spoilage. The harmful bacteria spreads on food and multiply in favorable conditions. Bacterial multiplication takes place in many ways especially the food handlers who are involved in the food processing chain, make safe food into hazardous to eat. Other sources like raw food, including meat, poultry, fish, and water especially when polluted with sewage or animal feces, insects, and rodents (Argudin *et al.,* 2010).

1.4.1.1.1 Factors Affected microbial survival

There are some factors that affect the bacteria to survive. Type of food is one of these factors; high protein food such as meat are particularly vulnerable to biological contamination from bacteria, so they are considered high risk foods. Water also is essential for bacterial growth, so drying food is a way of food preservation. In addition, Oxygen is an important factor; aerobic bacteria need air to survive, while anaerobic can survive oxygen lack, which may cause food poisoning in canned food. Also, the pH level; Bacteria thrive in neutral foods that have pH level of 6-8.9. Meat and sea food are examples of neutral food. Furthermore, the time and temperature; for bacteria to multiply to a dangerous level it needs time and right temperature. Maximum growth is achieved when temperature is between 5^{o}c – 60^{o}c (danger zone) Banwart (1989). High risk foods are those have ideal conditions for growth, they are neutral in acidity, starch or protein, moist food, such as seafood, cooked rice or dairy.

Low risk foods usually are high in acidity, high in salt or sugar, dried, canned or vacuum packed, like pickles, uncooked rice, however they still need appropriate handling (Banwart, 1989).

1.4.1.2 Chemical Contamination

Contamination occurs when food comes in contact with chemicals and can lead to chemical food poisoning. Sources of contamination includes kitchen cleaning agents, unwashed fruits and vegetables; pesticides and fungicides used in agriculture are harmful if consumed without washing, food containers made from non-safe plastics; are not designed for multiple use, pest control products; fly spray and rodents poisons are extremely hazardous if consumed, and chemicals used in equipment maintenance; some kitchen machines need regular oiling (Banwart, 1989).

1.4.1.3 Physical Contamination

Happens when actual objects contaminate food and they may be source of biological contamination as they may harbor dangerous bacteria like fingernails.

Common sources of physical contamination are hair, Glass or metal; cracked or broken utensils should be thrown away, pests; such as mice, rats and cockroaches leave droppings, flies and insects can also come in contact with food, dirt; they are so small, so unnoticeably contaminate food, they can get in food from unwashed food and vegetables, and also Fingernails; keep nails short and clean and avoid wearing fake nails (Al-Bahry *et al*., 2014)

1.4.1.4 Cross Contamination

Accidental transfer of contaminants from one surface to another usually from improper handling. In food, it refers to transfer of contaminants from surface object or person to food. Cross contamination usually of biological type, but can be also physical or chemical. Sources of cross contamination are clothing; dirty clothes can transfer bacteria from one place to another, utensils; separate utensils should be used to prepare different types of food, personal hygiene; coughing,

sneezing, touching face and hair before food handling, and washing hands is essential, pests; flies, rodents, and cockroaches carry harmful bacteria which can be transported to different places, thus Pest control policy should be applied, raw food storage is one of the most common types of cross contamination; when raw food comes in contact with ready to eat food, and waste control; garbage should be sealed and stored away to prevent contamination (Banwart, 1989).

1.4.1.5 Preventing Food Contamination

There is a great impact of these pathogens to the health and welfare of pets. The risk increases in pathogens shedding by pets and its effect on high-risk populations such as hospitalized animals, kenneled pets, puppies and geriatrics, in addition to the veterinary health care. The best way of prevention is through food safety training and education. Food handlers need training in important food safety concepts and practical knowledge, such as: safe cooking temperature, proper storage of high-risk food (potentially hazardous food), sanitation and sound management and personal hygiene with relevance to food hygiene (AIFS, 2016).

1.5 Bacterial Contamination in Pet Processed Food

Bacteria and other microorganisms contaminate pet processed food and cause digestive tract disease. Most pet foods are exposed to potential sources of microorganisms during production, handling, processing, storage, preparation or distribution for consumption. This contamination can be bacteria in water, air, feed or fertilizer, animal and human beings, processing equipment, ingredients and packing materials (Blumenthal, 1990).

The load of bacteria depends on original contamination, increases or decreases during processing, recontamination of processed product, and growth during storage and handling (Banwart, 1989). The usual number of bacteria in most

animal products used for food is 1,000 to 10,000/gram. Ground meat is more contaminated than cuts because of the type of meat used and handling during grinding. Low bacterial count is seen in heated food however, poor sanitation, recontamination, or poor handling and storage cause some heated products to have higher bacterial count. Bacterial contamination is common reason for food rejection. In recent study to determine the number and kind of bacteria that could be cultured from many commercial dry pet food (Cullor, 1995), it was surprising that all such foods are contaminated with bacteria. Rapid bacterial multiplication is seen in dry food moistened for puppies causing severe vomiting and diarrhea.

1.5.1 *Salmonella*

Based on the fact that raw meat for human or animal consumption and different bacterial hazardous contaminants are inseparable (LeJeune and Hancock, 2001; Rose *et al*., 2002; Woteki and Kineman, 2003)**,** *Salmonella* have become the first concern and are best documented as the cause of diarrhea in dogs. *Salmonella* can be cultured from the feces of up to 30% of dogs (Borland, 1975), many are normal with no disease signs. Infection usually follows ingestion of contaminated food or water, the food of animal origin e.g., poultry and their products are most frequent sources. The result of FDA survey of vegetable and animal protein ingredients used in animal feed shows 57% of animal protein samples and 36% of vegetable protein samples are positive for *Salmonella*. In other data more than 60% and 37% tested positive respectively. Cooking pet food is used to kill *Salmonella, but* contamination may occur from the processing plant even after cooking.

In a survey in North America, generally, frozen raw pet food has proportion of *Salmonella*-positive samples ranging from 7.1% (Strohemeyer *et al*., 2006), 8% (Nemser *et al*., 2014), 9% (Mehlenbacher *et al.*, 2012), 20% (Weese et al., 2005) and 21% (Finley *et al.*, 2008). In contrast, just one of 480 (0.2%) processed dog

food yielded *Salmonella* in a study from USA (Nemser *et al*., 2014). Monitoring of *Salmonella* contamination in pet food by APHA (American Public Health Association) in UK reported isolations from raw versus processed food in ratios of approximately 6:1 in 2015 and 20:1 in 2016, despite all samples coming from larger food sectors (APHA, 2017).

The frequency of shedding *Salmonella* in feces by dogs fed raw food correlates with the *Salmonella* risk of the food material in many studies (Joffe and Schlesinger, 2002**;** Finley *et al*., 2007; Lefebvre *et al*., 2008; Lenz *et al*., 2009; Kantere *et al*., 2016). Raw feeding is supposed to be a major risk factor of *Salmonella* shedding. It occurs at a similar or higher frequency than the ingestion of known contaminated pet food (Lenz *et al*., 2009; Leonard *et al*., 2011) suggesting that *Salmonella* ingestion leads to chronic shedding in dogs. This concept is supported when shedding is noticed for 1 to 11 days after 1 day of feeding Salmonella-contaminated commercial raw food (Finley *et al.*, 2007).

Diarrhea is not a typical feature of *Salmonella*-shedding dogs (Brisdon et al., 2006; Finley *et al*., 2007; Reimschuessel *et al*., 2017), although clinical salmonellosis has been reported in association with raw feeding (Morley *et al*., 2006).

1.5.2 *Staphylococcus aureus*

It is considered as the second most common cause of food borne bacterial disease found in the contaminated meat. It produces toxins cause vomiting and diarrhea. Exotoxins damage the intestinal mucosa and enterotoxins stimulate the intestine to secret large amount of fluids that cannot be reabsorbed. *Staphylococcus aureus* produces various toxins. Staphylococcal enterotoxins are family of nine thermostable enterotoxins serotypes of family pyrogenic toxins

(superantigens). These toxins can cause immunosuppression and non-specific T-cell proliferation. They are stable, and resist high temperature and conditions of drying and freezing. They are also resistant to proteolytic enzymes (Pepsin and Trypsin) at low pH enabling them to function in the digestive tract after infection (Zaghloul, 2015).

Enterotoxins act directly on the intestinal epithelium and the Vagus nerve, stimulating the emetic center. Only 0.1 ug of the toxin can cause poisoning in humans. It can also cause toxic shock syndrome due to production of toxic shock syndrome. *Staphylococcus aureus* can also cause toxic shock syndrome due to the production of the Toxic Shock Syndrome Toxin 1 (TSST-1) and Enterotoxin Type B (Nyenje *et a.,l* 2013; Spaulding *et al*., 2013; Zaghloul 2015). Food-borne illness due to *S. aureus* can be prevented, as the permissible temperature for growth and production of enzymes is between 6^0c and 46^0c, thus cooking above 60^0c and refrigerate below 5^0c to prevent microorganism's growth. Therefore, by good manufacturing and hygiene practices can avoid contamination (Zaghloul, 2015). Food processors are said to be the important source of contamination. Adequate hygienic measures should be applied, including contaminated handlers, refrigeration of food. Contaminated food can be cooked to destroy bacteria, but toxins when formed cannot be destroyed by heating.

1.5.3 *Clostridium perfringens*

Ranks third most common bacteria causes food-borne illness and well documented in dogs and cats (Anonymous, 1995). It is normal inhabitant in small intestine of small animals and cause no problems. It is a gram-positive anaerobic spore-forming bacillus. It is classified to five strains (A -E) based on production of four major toxins; alpha, beta, epsilon and iota (Marks and Kather, 2003).

Clostridium perfringens type A is recovered from both intestinal tract of animals and the environment, while type B, C, D and E are less common in animals. The enterotoxin (CPE) is commonly associated with type A strains.

A disruption of the normal microflora of the gastrointestinal tract can lead to increase in the concentration of *C. perfringens* followed by sporulation and release of enterotoxin. This might be due to dietary changes, stress or coinfection with another pathogen. One study showed that dogs fed high protein diet had increase in the fecal counts of *C. perfringens* enhancing enterotoxin production (Steen *et al.*, 1997).

Commercial pet food can be cooked to kill the organism, but the spores are still present to cause illness as they are resistant to heat. It is common to find enterotoxigenic *Clostridium perfringens* in dogs with gastrointestinal disease and can be responsible for chronic diarrhea. Testing is by examining fecal samples for the spores and identifying its enterotoxin in feces.

Clostridium perfringens infection usually linked to environmental contamination those results in transmission of enterotoxin-producing strains. Many cases develop after boarding at a kennel or during hospitalization. It is unknown whether they get infected from premises or contaminated food (Twedt, 1993).

1.5.4 *Escherichia coli*

It is found in the large intestine of normal animals causing no harm. Some are not pathogenic, others can invade the intestinal mucosa and the body producing an enterotoxin or an exotoxin that destroys the mucosa and cause hemorrhagic diarrhea (Strombeck and Guilford, 1990). Some subtypes of *E. coli* are pathogenic; producing certain colonisation factors and toxins. The shiga toxin-producing *E. coli* (STEC; of serovar O157:H7) is known in the human field. Heating reduce

their ability to cause gastrointestinal upsets by killing the organism and destroying some of the toxins, but some toxins are heat resistant.

Escherichia coli in food represents "fecal contamination" and is common for some pet food ingredients than in human food (Hollingsworth and Kaplan, 1997). Meat meals found in many pet foods are prepared from dead animals are very contaminated with Coliforms. High prevalence values of *E. coli*-positive samples are found in commercial raw pet food as compared with processed food (Strohmeyer *et al*., 2006; Freeman *et al*., 2013), so it is important to feed pet foods made only from "wholesome" foods.

1.5.5 Other bacteria

Other types of bacteria with ability to cause gastrointestinal diseases include ***Bacillus cereus, Campylobacter jejuni, Streptococcus*** species and others with unknown importance.

1.6 Types of Bacterial Toxins

A bacterial toxin is a macromolecule of protein origin, cause toxic damage in specific organ of the host (Iriarte *et al.*, 2001). Toxins can be divided into Endotoxins and Exotoxins.

1.6.1 Endotoxins or Lipopolysaccharides (LPS):

Endotoxins or Lipopolysaccharides (LPS) are component of the outer membrane of the Gram-negative bacteria, it is the most important antigen of the bacteria. They are released into the medium after some processes as lysis and cell division, it is capable of causing enterotoxin shock and tissue damage. (Romero Hurtado and Iregui, 2010).

1.6.2 Exotoxins

Exotoxins are macromolecules of protein origin, which are produced and then released to the medium by the microorganisms (Hernández-Cortez *et al*., 2017).

1.6.3 Types of Exotoxins

1.6.3.1 Toxins Type I

It modifies the host's cell without internalizing in the cells, like superantigens produced by *Staphylococcus aureus* or *Streptococcus pyogens* (Hernández-Cortez et al., 2017).

1.6.3.2 Toxins Type II

Toxins like haemolysin and phospholipases. These toxins are characterized by pore formation and/or destroying the cell membrane and pathogen get access to the host cell (Argudin *et al.,* 2010).

1.6.3.3 Toxins Type III

Known as A /B due to their binary structure. Fraction B binds to the cell receptor and fraction A possesses the enzymatic activity. For example, the shiga toxin produced by *E. coli* O157:H7, Cholera toxin (Ctx) by *Vibrio cholera*, and Anthrax toxins produced by *Bacillus anthracis* (Ramachandran, 2014).

Exotoxins of Gram-negative enteropathogenic bacteria play important role in the pathogenesis of diarrheal diseases causing hypersecretion of liquids without destruction of intestinal mucosal cells. They are called Enterotoxins which are different from Cytotoxins (Sears and Kaper, 1996).

1.6.4 Toxins produced by pathogens involved in food-borne diseases

Cholera toxins (Ctx) (*V. Cholerae*), Thermolabile toxin (LT) and Thermostable toxin (ST) (enterotoxigenic *E.coli*), shiga-toxin (*Shigella dysenteriae* and *E. coli* O157:H7), Non haemolytic enterotoxin (NHE) (*S. aureus*), cytotoxin K or cyt K (*Bacillus cereus*), (Clostridium botulinum), (*Clostridium perfringens*) CPE Enterotoxin Alpha-toxin, Beta-toxin & Lofa-toxin (*Cl. perfringens*). Botulinum toxin (BTX); (*Clostridium botulinum (*Todar, 2012; Lindbäck and Granum, 2006)

1.7 The impact of the critical control points in the Dog Health and Welfare

1.7.1 Kennel Flooring

To ensure dog health and welfare, the floor chosen must meet some criteria, it should be safe, comfortable for lying, standing, walking and easy to clean and disinfect. Dogs spend more time with their feet in contact with urine and feces, even the daily removal of excrement, the porous kennel floor may be saturated with urine and dirt. The dog usually drags dirt into kennel making quite dingy and messy. Accumulation of feces on floors can cause a lot of infections and sickening of dogs, lead to spreading of infection to human habitation (Webb and Nilsson, 1983).

Some flooring materials may harbor bacteria and may be difficult to sanitize and lead to greater risk of disease. Oral transmission occurs by ingestion of infectious agents through drinking or eating contaminated water and food and oral contamination with surfaces like ground and floors.

Several common diseases of dogs are transmitted through feces and feces contaminated food, water and environment, some include viruses (Parvovirus, coronavirus), bacteria (*Salmonella, Campylobacter*), Protozoa (*Giardia, Coccidia*),

and intestinal parasites (tapeworms. hookworms, roundworms). They can remain infectious for long time in the environment (Newbury *et al.*, 2010**).**

1.7.2 Feeding Dishes (Bowles)

Dog feeding bowls are breeding ground for dangerous germs that put human and animal health at risk. Potentially fatal bacteria including *E. coli*, *Salmonella* and *S. aureus* (MRSA) had been isolated from different types of bowls. Feeding bowls are said to be the third most contaminated item in the household. The increase of contact between human and dogs is a cause of concern for transmission of the bacteria.

Researchers from Hartpury University in UK, conducted study to identify whether the material; plastic, ceramic or stainless steel, and length of use of dog bowls influences the quantity and species of bacteria present (Wright and Carroll, 2018). The study by Carroll and Wright for six weeks for several sets of bowls. The dogs were healthy and the bowls were brand new and sterilized prior to the investigation. After use, the bowls were swabbed for several times over the six weeks for bacterial identification.

Their result showed highest number of bacteria in plastic bowls, but the harmful pathogens, including *E. coli* and Methicillin Resistant *Staphylococcus aureus* (MRSA) exploded in the ceramic bowls. At first, the researchers thought that bacteria would thrive in the plastic bowls, as plastic wears away over time leaving grooves and ridges which help bacteria adhere to the surface making cleaning less effective. They are shocked by the result of the ceramic bowls, they explained that as the ceramic enables bacteria to form structure called biofilms, which allow large number of bacteria to adhere and colonize surfaces as a group and protect them from certain elements that would normally kill bacteria. Biofilm appears in

many colors, including red, green, pink, yellow, purple, orange, brown, colorless or black. It also creates a putrefied smell. They are life-threatening when ingested by human or pets like: *Serratia marcescens* (the pink film you see in bowls, shower curtains, and other wet areas*),E. coli, Candida albicans, Chlamydia pneumoniae, Borrelia burgdorferi* (Lyme disease), *Clostridium difficile* (the most common cause of human GI infection and a growing epidemic), *Helicobacter pylori* (causes human stomach ulcers and gastritis), *Klebsiella pneumoniae, Legionella pneumophila, Listeria, Staphylococcus aureus, and Staphylococcus epidermidis (* Zambori *et al.*, 2013).

The stainless-steel bowls had far less germs. The silver in the stainless steel had been seen to have antimicrobial properties which matches their results. From this study, stainless steel is preferred material to use to limit the number of bacteria that colonize on the bowls. Regardless the material, bowls are fomites; which are objects that can transmit bacteria from dog to human or human to dog. In a group of dogs, bowls are more likely have large variety of bacteria present, however, the quantity varies depending on many factors like frequency of use, size of animals, position of the bowl in the kennel, and cleaning regime. Therefore, it is hard to determine the number of animals would have impact (Wright and Carroll, 2018).

1.7.3 Pet food

Pet food safety represents a substantial challenge over the traditional food safety as hazard have an impact on the animals and indirectly affects human who share its environment. Chemical contaminants in pet food have direct impact on animal health. In 2007, cases of kidney damage and failure was reported in USA due to melamine and cyanuric acid (FDA, 2007; WHO, 2009). During 2005 -2006, more than 100 dog deaths due to high aflatoxin level, results in large recall of the product (FDA, 2005).

This shows the influence of improper ingredients in the safety of the product. The impact of microbiological contamination of pet food has on animal health is less clear because few of the potential outbreaks are investigated.

Improperly canned dog food has similar risk of botulism due to *Clostridium botulinum* toxin as for human food. Likewise, while adult dogs are less likely to be symptomatic than human, *Salmonella enterica* in dogs is common, and salmonellosis can be serious in puppies or elderly dogs (Galton *et al*., 1952; LeJeune and Hancock, 2001).

Pet food can be source of pathogenic micro-organisms that can have impact on pet owners' health, they act as a vehicle for foodborne pathogens resulting in direct or indirect transmission, like handling of contaminated pet food by owners or when the dog is asymptomatically infected and serves as reservoir for pathogenic microorganisms either through direct contact between the dog and the owner, or indirectly through fecal contamination of the environment.

Outbreaks of human salmonellosis have been linked to contamination of dog pet food and treats like pig ears (Center for Disease Control and Prevention, (CDC, 2006; CDC, 2008).

The U.S. Food and Drug Administration coordinated with the U.S. Centers for Disease Control and Prevention (CDC) and state agencies to investigate human cases of salmonellosis related to several *Salmonella* serotypes linked to exposure to pig ear pet treats from Argentina, Brazil, and Colombia. FDA continues to carry out a public health investigation into the issue of pig ears contaminated with Salmonella (FDA, 2019).

Treats are products typically given by dog owners to please their pets, or as training rewards. Such as all chews, bones, toys and exercisers made of animal

skin, hide, wood or manmade materials, hooves, ears animal bones, ligaments (AAFCO, 2012).

Managing *Salmonella* contamination in the production of pet food can be very challenging because the raw materials used (grain, meat, and poultry) may be naturally contaminated, this is why risk assessment should be applied to detect the material or process causes the risk, and develops a control plan for product safety. The increased attention about pet food safety, is increasing emphasis on being able to rapid evaluation and identification of chemical and microbiological contamination. The pet food industry is responding rapidly to maintain the consumers' confidence in product class which have direct impact on health and well-being of dogs (Buchanan *et al.*, 2011).

1.7.4 Handlers hands

Improper hand washing Transfer biological hazards to animal food through improper handling or maintenance practices like not applying cleaning and sanitizing measures for the animal food-contact surfaces. Handlers may transfer biological hazards from one point to another even by their clothes or shoes. (FDA, 2018**)**. Food handlers are playing an important role in food-borne illness, during handling raw materials processing or by contaminating the final product. Food handlers are the main source of food contamination via direct contact like *Staphylococcus aureus* which is usually present in people's nasal passage, throat or skin. The contamination can occur via hands or respiratory secretions. Time and temperature abuse of contaminated food results in growth of the *S. aureus* and enterotoxin production in food.

Handlers also may be incriminated in food-borne Salmonellosis when cross contamination occur during food handling and poor hygienic measures and

temperature control practices. This happens when eating contaminated raw food without further processing like cooking (FSANZ, 2018).

1.8 Public health concerns associated with dog food

Food safety draws attention of public and being of an important concern to veterinary profession, as dogs are susceptible to a large number of food-borne infections, which is of major importance to public health due to the risk of zoonotic infection. Dogs can transmit several zoonotic diseases to their owners, this why the owners should be informed about the modes of transmission to reduce the risk of infection. *Salmonella* is a bacterial pathogen received most attention for its high risk. In one study, more than half of strains of *Salmonella* spp. identified in feces of dogs matched the strains found in their diet. Dogs may be subclinical carriers following exposure. Pet-to-person transmission may occur if infected dogs are handled without proper hygienic practice, and the handling of Salmonella- positive food is a well-established risk factor for human salmonellosis (Cobb and Stavisky, 2013), however, contact with pets has also been identified as a route or a risk factor for human salmonellosis in several case reports and studies (Finley *et al.*, 2006; Domingues *et al*., 2012; Freeman *et al*., 2013), indicating that pets which have consumed Salmonella- contaminated feed also pose an infection risk to owners.

The most likely routes of transmission to pet owners when the food is contaminated, during food preparation and clearing up meals, licking, and from *salmonella* shed in feces. It is ineffective to use domestic cleaning in disinfection of feeding bowls contaminated with *Salmonella* (Weese and Rousseau, 2006). In Raw-feeding kennels, *Salmonella* in contaminated surfaces was prevalent despite the proper cleaning routine (Morley *et al.*, 2006).

The human clinical disease associated with raw-fed pets is likely to occur sporadically rather than in outbreaks. In view of risk of human infection, some public health bodies advise on the safe handling of raw pet food to alleviate sick risks (FDA, 2018; CDC, 2008).

Escherichia coli is a commensal enteric species and strains may be transferred between dogs and owners (Naziri *et al*., 2016). Higher counts of *E. coli*-positive samples have been found in commercial raw-pet food when compared to conventionally processed food (Strohmeyer *et al.*, 2006; Freeman *et al*., 2013).

Some subtypes of *E. coli* are pathogenic develop certain colonization factors and toxins. The Shiga Toxin Producing *E. coli* (STEC) of serovar (O157:H7) are seen in human field. In a survey conducted in the Netherlands, it was isolated from nearly 20% of some raw diets (Van Bree *et al.*, 2018). However, Serogroup O157 was not isolated from 616 samples in two studies in the USA (Lenz *et al*., 2009; Nemser *et al*., 2014). This difference indicates the variation in the local meat contamination and types of source of meat.

A recent investigation in UK, identified close related (STEC) O157 isolates from four human cases, three are linked with dogs on raw diet (Byrne *et al.*, 2018).

Other food-borne bacterial agents that can pose risk to pets, with little or unknown risk of secondary transmission to humans from pets. *Clostridium botulinum* toxin can cause neurological effects on dogs, some packaged food is capable of promoting the growth of *C. botulinum* and toxin production. The food should be fully cooked to destroy the toxin before feeding (Byrne *et al.*, 2018).

Staphylococcus aureus and *Bacillus cereus* are toxin-producing organisms, can be found in raw meat and commercially prepared food. They can produce toxins if

the food is left for many hours before eating. (Gareis and Walz, 1994; Balzaretti *et al*., 1985).

In case of *S. aureus*, food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of contamination by direct contact or through respiratory secretions (Argudín *et al*., 2010).

1.9 HACCP system

HACCP system is the systematic preventive approach to food safety. It is a tool to assess hazards either physical, chemical or biological and establish a preventive system than the end product inspection. The objective of HACCP is making the product safely and being able to prove it (FEDIAF, 2018).

1.9.1 Develop of HACCP Concepts

HACCP concepts were pioneered in 1960 by Pillsbury Company to assure the safety level required by NASA for food produced for space program.

The use of HACCP principles in the promulgation of regulations of low acid canned foods was completed in 1974 by the United States food and Drug Administration (FDA) (Abdulla, 2010*).* HACCP principles were adopted by other major companies in 1980.

HACCP has been used for decades to evaluate and control safety hazards in human foods and now being used to make improvement in pet food safety. In USA, the FDA launched a Reportable Food Registry requiring pet food companies to report incidents of adulteration with HACCP as the best approach to pet food safety.

The codex Alimentarius is internationally recognized food and hygiene standards of which HACCP is one standard which are published in the Codex. These voluntary global references will be enforceable when accepted as national standards by the member countries (FEDIAF, 2018).

Recognizing the importance of HACCP to food control, the twentieth session of the Codex Alimentarius Commission, held in Geneva, Switzerland from 28 June to 7 July 1993, and adopted Guidelines for the application of HACCP system (ALINORM 93/13A, Appendix II). The commission was also informed that the draft revised General Principles of Food Hygiene would incorporate the HACCP approach. The revised Recommended International Code of Practice - General Principles of Food Hygiene [CAC/RCP 1-1969, Rev 3 (1997)] was adopted by the Codex Alimentarius Commission during its twenty-second session in June 1997. The (HACCP) system and guidelines for its application is included as its Annex (FAO, 1998).

1.9.2 Advantages of HACCP

The HACCP system when applied to food safety management uses the approach of controlling critical points in food handling to prevent food safety hazards, identifies specific hazards and measures for their control to ensure the safety of food and to prevent and reduces the reliance on end product inspection and tests.

The system can be applied throughout the food chain from primary production to the consumer. A properly implemented HACCP system leads to greater involvement of food handlers in understanding and ensuring food safety.

HACCP enhances the responsibility and degree of control at the level of the food industry. The application of HACCP system can aid inspection by food control regulatory authorities and promote international trade by increasing buyer`s confidence (Abdulla, 2010).

The HACCP system offers a structured approach to the control of hazards in food processing and properly applied, identifies areas of concern, and appropriate control measures before product failure is experienced (Jervis, 2002).

1.9.3 HACCP Principles

1.9.3.1 Principle 1 - Conduct a Hazard Analysis

The application of this principle involves listing the steps in the process and identifying where significant hazards are likely to Occur.

1.9.3.2 Principle 2 - Identify the Critical Control Points

A critical control point (CCP) is a point, step or procedure at which control can be applied and a food safety hazard can be prevented, eliminated, or reduced to acceptable level.

1.9.3.3 Principle 3 - Establish Critical Limits

A critical limit (CL) is the 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 a food safety hazard.

1.9.3.4 Principle 4 - Monitor CCP

The HACCP team will describe monitoring procedures for the measurement of the critical limit at each critical control point.

1.9.3.5 Principle 5 - Establish Corrective Action

Corrective actions are the procedures that are followed when a deviation in a critical limit occurs. The HACCP team will identify the steps that will be taken to prevent potentially hazardous food from entering the food chain and the steps that are needed to correct the process (FEDIAF, 2018).

1.9.3.6 Principle 6 – Verification

Those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan like auditing of CCP's, records prior, instrument calibration, and product testing (FEDIAF, 2018)

1.9.3.7 Principle 7 – Record keeping

Recording information that can be used to prove that a food was produced safely.

Chapter Two

Materials and Methods

2.1 Area Study

The study was conducted at Police Dog Unit, Al Ain, Abu Dhabi, United Arab Emirates, during period from December, 2018 to January 2019 . The swabs were collected from Dogs' Kennels during period of ten weeks.

2.1.1 Collection of Samples

Total of 300 swab samples were obtained from dogs' kennels, 30 swabs were taken weekly from the kennel, precisely from five critical points CCPs: Kennel floor, bowls before meal, Bowls after meal, handlers' hands, and canned food.

The kennels floor was made up of concrete tiles. Feeding Bowls were round shape and made of stainless steel. The canned food was purchased from known company. The 30 samples were repeated and taken from the same five stages CCPs from the kennel for 10 consecutive weeks.

The swab samples were collected in sterile tubes and preserved in cooled container and transferred to Microbiological laboratory for analysis.

The targeted bacteria in this study were: *Staphylococcus aureus, Salmonellae, Escherichia coli,* and *Clostridium perfringens.*

2.1.2 Collection of swab samples for microbiological testing

After wearing the suitable gloves, selecting a sampling area of about 10 cm X 10 cm (or 20 cm x 20 cm), then to break the seal round the tube containing the swab. After that, removing the swab from the tube and rubbing and rolling it firmly

several times across the sampling area, and finally, returning the swab into the tube and labeling the sample.

2. 2. Media

2.2.1 Bacterial Culture

Isolates of test organisms were obtained from the five CCPs from kennels. The samples were cultured in Blood agar, MacConkey's agar (MCA), Brilliant green agar, Nutrient broth, Nutrient agar, and Xylose lysine deoxycholate (XLD) agar. The plates were incubated at 37c for 24 hours and stored at 4^0c till used (Barrow and Feltham, 1993).

2.2.1.1 Blood agar media

The medium enriched and bacterial growth medium, used was trypticase soya agar, enriched with 5% sheep blood for isolation and cultivation of wide variety of fastidious organisms.

Blood agar ingredients

Ingredients	**gm/litre**
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0

2.2.1.2 MacConkey's agar medium

The medium is selective and differential culture medium commonly used for the isolation of enteric Gram-negative bacilli (*Enterobacteriaceae*). For differentiation of lactose fermenting from non-lactose fermenting Gram negative rods. It is based on the bile salt-neutral red-lactose agar of MacConkey. Crystal violet and bile salts are incorporated to prevent the growth of Gram-positive bacteria and fastidious Gram-negative bacteria such as *Neisseria* and *Pasteurella*. Gram -negative bacteria can tolerate bile salts because of their bile-resistant outer membrane.

MacConkey's agar ingredients

Ingredients	**gm/litre**
Peptone per liter	20.0 g
Agar	15.0g
NaCl	5.0 g
Bile Salts	1.5g
Neutral Red	0.05g
Crystal Violet	1.0g

2.2.1.3 Xylose Lysine Deoxycholate (XLD)

This medium is selective and differential medium for the isolation and differentiation of Gram-negative enteric pathogens. Primarily used for *Salmonella* and *Shigella.*

XLD ingredients

Ingredients	**gm/litre**
Lactose	7.5g
Sucrose	7.5g
Sodium Thiosulphate	6.8g
L-Lysine	5.0g
Sodium Chloride	5.0g
Xylose	3.75g
Yeast Extract	3.0g
Sodium Deoxycholate	2.5g
Ferric Ammonium Citrate	0.8g
Phenol red	0.08g
Agar	15.0g

2.2.1.4 Brilliant green agar

It is classified as a highly selective medium for the recovery of salmonellae except for the typhoid and paratyphoid bacilli. Principles of the Procedure Brilliant green dye inhibits gram-positive bacteria and a majority of gram-negative bacilli. Phenol red serves as a pH indicator and yields a yellow color as a result of acid production in the fermentation of the lactose and/or sucrose in the medium.

Brilliant Green Agar Ingredients

Ingredients	gm/litre
Proteose peptone	10.0
Yeast extract	3.0
Lactose	10.0
Sucrose	10.0
Sodium chloride	5.0
Phenol red	0.08
Brilliant green	0.0125
Agar	12.0

2.2.1.5 Nutrient broth

General purpose medium is used for large variety of microorganisms without particular nutritional requirements.

Nutrient broth ingredients

Ingredients	gm/litre
Tryptone	15.0
Meat Extract	2.5
Sodium Chloride	5.0

2.2.1.6 Plate count agar (PCA)

Also called standard method agar (SMA**)**, a microbiological growth medium commonly used to assess total or viable bacterial growth of a sample.

Plate count agar ingredients

Ingredients	gm/litre
Peptone	0.5%
yeast Extract	0.25
Glucose	0.1%
Agar	1.5%
PH adjusted to neutral at25C.	

2.2.1.7 Mannitol salt agar medium (MSA)

Mannitol Salt Agar Ingredients

Ingredients	**gms / Litre**
Pancreatic Digest of Casein	5.0 gm
Peptic Digest of Animal Tissue	5.0 gm
Beef Extract	1.0gm
Sodium Chloride	750.0gm
D-Mannitol	10.0gm
Phenol Red	0.025gm
Agar	15.0gm
Total	111.025gm

Distilled Water = 1000 ml /Final pH 7.4 ± 0.2 at 25°C.

All samples were cultured in different media. The total viable count (TVC) of the contaminates was carried out according to the methods of Miles and Misra (1938). The biochemical tests were performed for identification of isolated bacteria by an automated microbial identification system called Vitek 2.

2.3 Bacterial Counts

2.3.1 Total Viable Count (TVC)

The total viable count of isolated microorganisms was carried out using serial dilution to each sample as per (Harrigan and McCance, 2014).

Total Viable Count (TVC) is of one of the most common methods to determine cell number; the sample to be counted is diluted in a solution that will not harm the microbe and not supporting its growth. It is a quantitative estimate of the concentration of microorganisms such as bacteria, yeast or mold spores in a

sample. The count represents the number of colony forming units (CFU) per gram (or per ml) of the sample.

Total Viable Count (TVC) is achieved by plating serial tenfold dilutions of the sample until between 30 and 300 colonies can be counted on a single plate. The reported count is the number of colonies counted multiplied by the dilution used for the counted plate. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample), and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony forming units (CFUs). The idea is that each viable bacterium is separate from others and can develop a single colony (CFU), so the number of colonies represents the number of bacteria that can grow under the incubation conditions.

A wide series of dilutions (e.g.10^{4} to10^{10}) is normally plated on a Petri dish contained plate count agar, because the exact number of bacteria is unknown. Once the concentration of cells at the specific dilution was the determined the concentration in the original sample can be calculated by dividing into the original dilution.

The CFU/ml can be calculated using the formula:

CFU/ml = (no. of colonies x dilution factor) / volume of culture plate.

2.3.2 Miles and Misra method for Determining Bacterial CFU

Serial dilution of the suspension included bacteria was done in which, the dilution of 1x suspension was added to 9x of diluent. In case of unknown sample quantity or unknown bacterial quantity, dilutions should be made to at least 10−8. The average of three plates was calculated. This was required to have greater assurance of results. All three plates were inoculated with each dilution.

The samples were transferred to a nutrient broth test tube, then **5** ml of the solution is incubated at 37^0C for 18-24 hours (overnight) for bacterial growth. Firstly, serial dilutions prepared from the Normal Saline solution included bacteria to be diluted, then serial folds' dilution in sterile test tubes each contains **9** ml Normal Saline will be prepared. One ml of nutrient broth withdrawn by micro pipette and added to the first tube of 9 ml normal saline dilute **1**. From the first dilution 1/10 take 1 ml and add to the second tube of 9 ml normal saline the dilution is 1/100 repeat the process until reach 1/100000 concertation. From the 4^{th} tube (1/10000) using micro- pipette take 1 ml and spread it over the surface of Petri dish which contains Nutrient agar or count plate. Incubate overnight at 37^0c for 24 hours. The colonies will be counted after formation.

2.3.3 Colony Isolation

According to Cultural Media preparation (Miles and Misra 1938)

2.4 Identification

Vitek 2 is the mean of bacterial identification, it was an automated microbial identification and antimicrobial susceptibility system that provide of highly accurate and reproducible results. With its colorimetric reagent cards and associated hardware and software advances the VTEK 2 offers a state of the art of technology platform for phenotypic identification methods. Uses advanced colorimetric technology to determine individual biochemical reactions contained in a variety of microbe identification cards.

After incubation with standardized suspension of the unknown organism, each self-contained card is incubated and read by the instrument internal optics. Comparison of results to known species specific reaction in the Vitek2 database yields. The result usually within 4-6 hours. The reagent cards have 64 wells

contain an individual test substrate, which measures metabolic activities such as acidification, alkalization, enzyme hydrolysis and growth in presence of inhibitory substance. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixture. Each card has a pre-inserted tube for inoculation. Cars have bar codes contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading of the card onto the system.

There are currently four reagent cards for the identification of different organism classes; I/ GN- Gram-negative fermenting and non-fermenting bacilli, II/ GP- Gram-positive cocci and non-spore forming bacilli, III/ YST- yeast and yeast-like organisms, and IV/ BCL Gram-positive spore-forming bacilli.

Chapter Three

Results

Contamination at the points of bowls after meal was 37.60% with mean count of 5.05 cfu/cm^2, and kennel floor was 31.31%, with mean count of 4.21 cfu/cm^2, and low counts were detected in the canned food with 5.88% and mean value of 0.78 cfu/cm^2, and handlers' hands (6.26%) mean count of 0.89 cfu/$cm^{2.}$ (Table 1)

Table 1: Mean and standard Deviation, Standard Error and Percentage of Total 1: Viable Counts of Bacterial Contamination ((log^{10}cfu/cm^2) at Different Stages of dogs (300) Feeding Process in AlAin police K9, (Abu Dhabi Emirate)

CCPs	Mean (log^{10}cfu/cm^2) ±STD.DEV	Standard Error	Significant Difference	Percentage
Kennel Floor	4.21 ±0.75	0.09	* *	31.36%
Bowls Before Meal	2.55 ±0.27	0.35	* *	18.90 %
Bowls After Meal	5.05 ±0.46	0.05	* *	37.60 %
Handlers Hands	0.89 ±0.05	0.00	* *	6.26 %
Opened canned Food	0.78 ±0.10	0.13	* *	5.88 %

Isolation and Identification of Bacteria

Only two types of bacteria were isolated and identified at the five stages of feeding process and these were *Staphylococcus aureus*, and *Escherichia coli*.

Table (2) illustrates the concentration (%) of *E. coli* at the different stages of feeding process. High load of contamination by *E. coli* was detected at kennel floor (60.05%) and at the bowls after meal (34.46%). Neglected load was seen at the points of canned food and handlers' hands.

Table 2: Evaluation of of *E. coli* (%) at Different Stages of dogs (n=300) feeding process in AlAin police K9, (Abu Dhabi Emirate)

CCPs	Percentage %
Kennel Floor	**60.05%**
Bowls before meal	**3.37%**
Opened canned Food	**0.06%**
Handlers hand	**0.06%**
Bowls after meal	**36.46%**
Total	**100%**

Table 3: Evaluation of *Staphylococcus aureus* (%) at Different Stages of dogs (n=300) feeding process in AlAin police K9, (Abu Dhabi Emirate)

CCPs	Percentage %
Kennel Floor	**25.54%**
Bowls before meal	**3.23%**
Opened canned Food	**0.00%**
Handlers hand	**0.34%**
Bowls after meal	**70.89%**
Total	**100%**

The obtained results indicated that *S. aureus* was the most contaminant bacteria than *E. coli* at the different stages. *E. coli* was mostly seen in high load at the kennel floor rather than other stages (Table 3, 4).

Table 4: Percentage of *Staphylococcus aureus* and *Escherichia coli* isolated and identified at the different stages of dog (n=300) feeding process in AlAin police K9, (Abu Dhabi Emirate)

CCP	*S. aureus*	*E. coli*	TOTAL
Kennel Floor	**24.87%**	**1.58%**	**26.45%**
Bowls before meal	**3.15%**	**0.09%**	**3.24%**
Bowls after meal	**69.03%**	**0.96%**	**69.98%**
Handlers hands	**0.33%**	**0.00%**	**0.33%**
Opened canned food	**0.00%**	**0.00%**	**0.00%**
TOTAL	**97.38%**	**2.63**	**100.00%**

The study shows a statistically significance difference at ($P \leq 0.05$) for the critical points.

Chapter Four

Discussion

In this study, a variety of contaminants were identified at the stages of the feeding processes, which displayed a highest bacterial viable count (TVC) at the kennel floor, and the Bowls after meal. These contaminants are supposed to be shed from the feces of dogs, their oral saliva, or nasal discharge, or from the dog handlers. *Staphylococcus aureus* and *Escherichia coli* were seen at lower mean bacterial count (TVC) at points of handlers' hands and canned food. The low or nearly neglected bacterial counts in the canned food is in accordance to the ICMSF (International Commission on Microbiological Specifications for Foods). Canned pet foods are terminally heat processed in hermetically sealed containers and are commercially sterile and subjected to the regulations for low-acid canned foods, and when in compliance are not of public health concern (Silliker and ICMSF, 1980). Intermediate wet pet foods and the dry kibbles are subjected to a heat process during extrusion and pelleting, which will destroy the vegetative cells of pathogenic bacteria. The prevention of recontamination following heating, then, it is the critical control step in their processing.

Matching of the results of pathogenic microorganisms in ready-to-eat food (RTE) with the standards of Compendium of Microbiological Criteria for Food (FSANZ, 2018), interpreting results of (cfu/g) for *S. aureus* is regarded as satisfactory if it is $<10^2$. Results of <3 for *E. coli* is satisfactory and of marginal hazard at the counts of 3- $<10^2$. It is of health concern to know that Shiga toxin producing *E. coli* (STEC) is potentially hazardous when detected in 25g of (RTE) food. This corresponds to this study as the canned food used for feeding is purchased from known sources using "wholesome" pet foods.

The handler's hands also showed a low level of bacterial count which clearly indicates that the standards of hygiene within the K9 facilities under investigation is satisfactory.

Staphylococcus aureus is a part of the normal microbiota in humans and animals. It is an opportunistic pathogen noted in clinically healthy individuals. Food handlers are main source of food contaminating microbes via direct contact as *S. aureus* is usually present in people nasal passages, throat, and skin. *Escherichia coli* is a bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some can cause serious food poisoning. Usually present when self-hygiene is not ensured. In agreement with study by Olsen *et al.* (2000*)* who reported that food service establishments are source of food-borne illness and food handlers contribute to food -borne illness outbreaks. Furthermore, according to World Health Organization (WHO) food handling personnel play a vital role in food safety through the chain of production to storage. One of the major threats of the food industry is that the contamination with food-borne microbes of human origin resulting from improper handling and processing. Handlers may be incriminated in food-borne illness when cross contamination occur during food handling and poor hygienic measures (Elena *et al*., 2018). The data in this study obtained from the kennel floor showed a noticeable count of both *S. aureus* and *E. coli,* so oral contamination of infectious agents occur through eating or drinking contaminated food, water, and oral contact with contaminated environmental surfaces such as ground of floor. *Staphylococcus aureus* is identified at this stage as it is commonly found on the skin of mammals, birds, fomites, and secretions from nasal passages and throat, this is in agreement with Al-Bahry *et al.* (2014).

The presence of *E. coli* in the kennel floor is attributed to the contamination by the feces as the bacteria is found in the large intestine of normal animals. This data is in accordance to the findings of study done by Stella *et al.* (2018) on how flooring substrate impact kennel and dog cleanliness in breeding facilities of 118 dogs housed on three different types of flooring. They found Thirty-one percent or fewer kennels have fecal contamination and culture-positive for *E. coli* after routine cleaning. The kennel flooring surfaces were swabbed and cultured for presence of *E. coli.* The Positive results ranged from 7% to a higher of 31% with an average of 23.7% of samples taken from kennels after cleaning.

These findings indicate that a well-managed kennel can maintain healthy dogs on different types of flooring substrate, but concrete flooring types can permit maintenance of dog cleanliness. Such flooring substrate is used in the Police dogs' facilities in study, though, standard cleaning protocols should be implemented to minimize Coliform recovery to promote dog physical health and hygiene and prevent cross-contamination Elena *et al*. (2018). The mean TVC obtained from the feeding bowls before and after meal revealed the identification of *S. aureus* and *E. coli* as such, with a considerable count in the bowls after meal, in accordance with the study and results done by Wright and Carrol (2018) from Hartpury University in UK, who found harmful pathogens, like *E. coli* and MRSA in plastic and ceramic bowls and less counts in stain-less bowls.

In another study by Abdel-moein *et al.* (2011) who found Methicillin-Resistant *Staphylococcus aureus* (MRSA) as an Emerging Pathogen of Pets in Egypt with a Public Health Burden diseases, who looked for enterotoxigenic *Staphylococcus* in 70 dogs and 48 pet cats. Swabs were collected from the mouth, nose and wounds, nasal swabs from 26 people. They isolated enterotoxemic *S. aureus* from 10% of dogs and 2.1% of cats, most of the positive results are from pets' oral samples,

indicating that dogs can pose a risk and potential source of *S. aureus* that can be incriminated in food poisoning, since it can be presumably shed in saliva.

Thus, the increased count in the bowls after meal may be attributed to shedding of saliva, as the oral dog flora contains different types of microorganisms including *E. coli* and *S. aureus*.

Another possible way of contamination is by the *S. aureus* on the skin and hair of dogs, and soiling of the bowls by dogs' own feces might be a source of *E. coli* contamination.

Although, bowls are fomites that bacteria attach to it, and transfer it to anything in touch, that way can spread the bacteria from dog to human and human to dog.

This study was faced by some challenges which can be summarized as, scarce of similar studies done, for debating the issues and comparing the results. The study was done in nearly ideal environment, where strict hygienic measures are implemented in the Police K9 unit facilities.

Conclusion

The results clearly showed that there was contamination at all stages of feeding process in the Kennel under study with variable counts. *Staphylococcus aureus* and *Escherichia coli* were isolated and identified at all stages. The highest contamination was seen at the bowls after meal and lowest at the handler's hands and the canned food. The standards of hygiene implemented in the facilities are relatively satisfactory.

Recommendations

1. Special emphasis must be given to raise the level of awareness for both dog handlers and workers in order to reduce the incidence of food-borne illness with special attention to some factors are included, thorough cleaning and disinfection of food and water bowls, proper handling and disposal of feces, personal hygiene with special concern to hands hygiene following contact with pet food, feeding bowls and feces.

2.leftover food must be promptly discarded and not allowed to stay in bowls, as some pathogens may lurks in these bowls, so a daily disinfection ideally with 10% bleach solution is recommended.

3. Recommendation to setup a HACCP system in police dogs' facilities to ensure health and welfare of the dogs.

4. More attention and further studies should be done in other Foodborne pathogens and zoonotic diseases which pose a great hazard for the dog handlers and workers and dogs' health.

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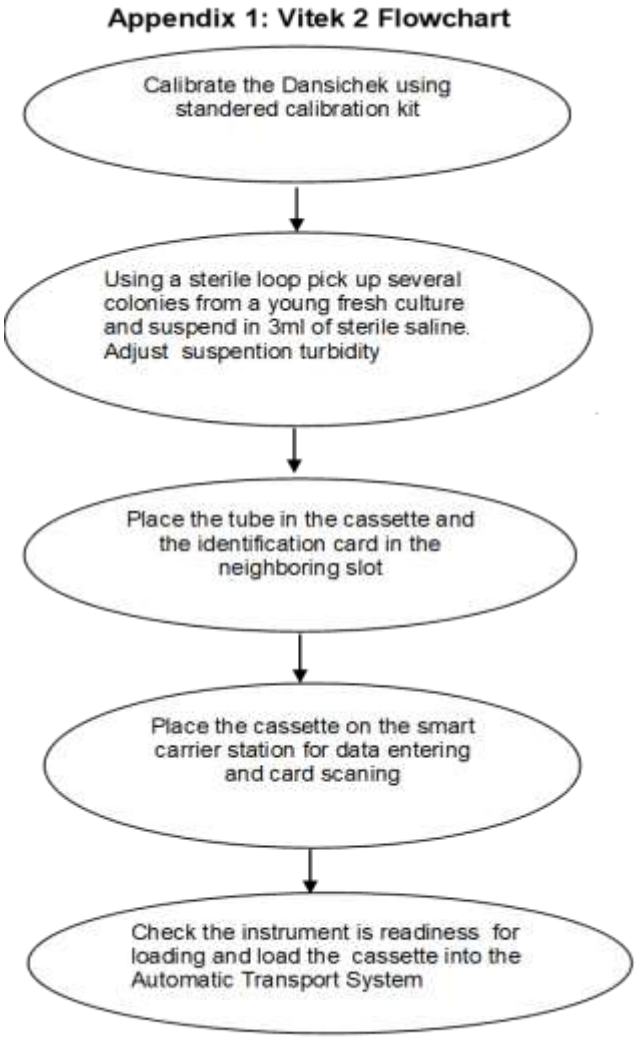
APPENDICES

Appendix 1

Flow chart shows the steps of identification by vitek-2 machine.



Appendix 2

Shows the full descriptive analysis of data obtained from the study for further and detailed clarification.

Descriptive Analysis of Data

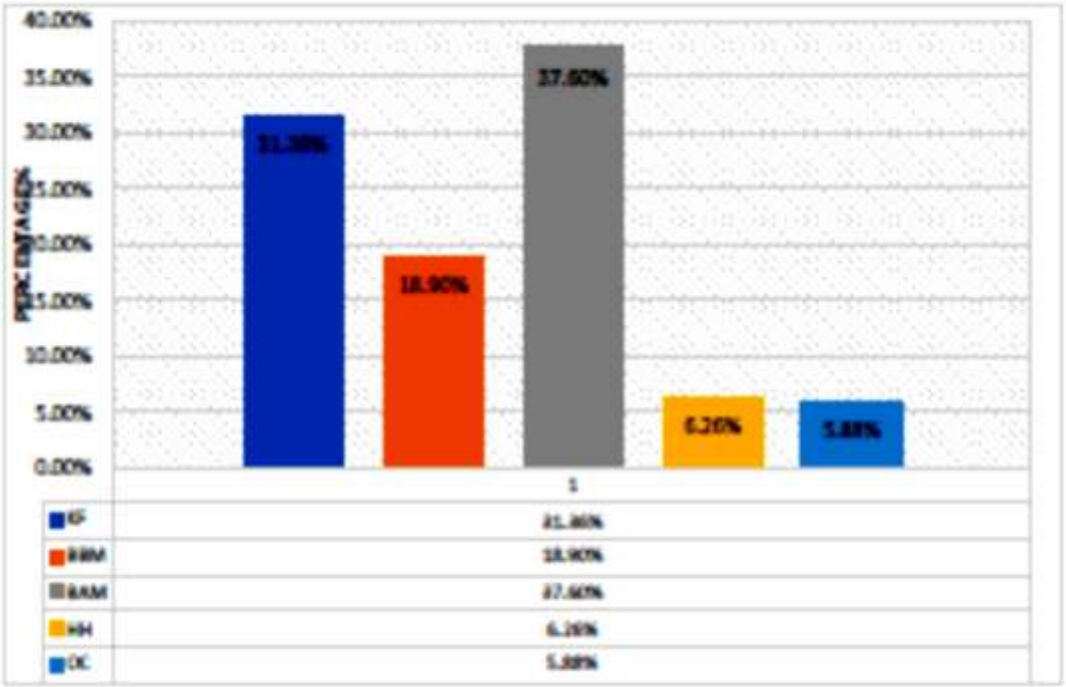
The total viable counts revealed several contaminants at the different points of feeding process rather than those intended for isolation and identification in this study (Table, 2).

The statistical analysis of data is obtained as shown in (Table 8) in forms of Mean (log10cfu/cm2), standard Deviation, Standard Error, and Percentage of Total Viable Counts of Bacterial Contamination.

A noticeable load of contamination at the points of Bowls after meal (37.60%) with mean count of 5.05, and Kennel Floor (31.31%), with mean count of 4.21, and low counts were detected in the canned food with (5.88%) and mean value of 0.78, and Handlers hands (6.26%) mean count of 0.89.

Table 1: Mean and standard Deviation, Standard Error and Percentage of Total Viable Counts of Bacterial Contamination ((log10cfu/cm2) at Different Stages of Feeding Process in dogs (n=300) in AlAin police K9, (Abudhabi Emirate)

CCPs	Mean ($log^{10}cfu/cm^2$) ±STD.DEV	Standard Error	Significant Difference	Percentage
Kennel Floor	4.21 ±0.75	0.09	* *	31.36%
Bowls Before Meal	2.55 ±0.27	0.35	* *	18.90 %
Bowls After Meal	5.05 ±0.46	0.05	* *	37.60 %
Handlers Hands	0.89 ±0.05	0.00	* *	6.26 %
Opened canned Food	0.78 ±0.10	0.13	* *	5.88 %



*KF: Kennel Floor * BBM: Bowls Before Meal * BAM: Bowls After Meal *HH: Handlers Hands * OC: Opened canned food

Figure (1) illustrates the Percentage of Total Bacterial Contaminants Distribution at the Stages of Feeding process.

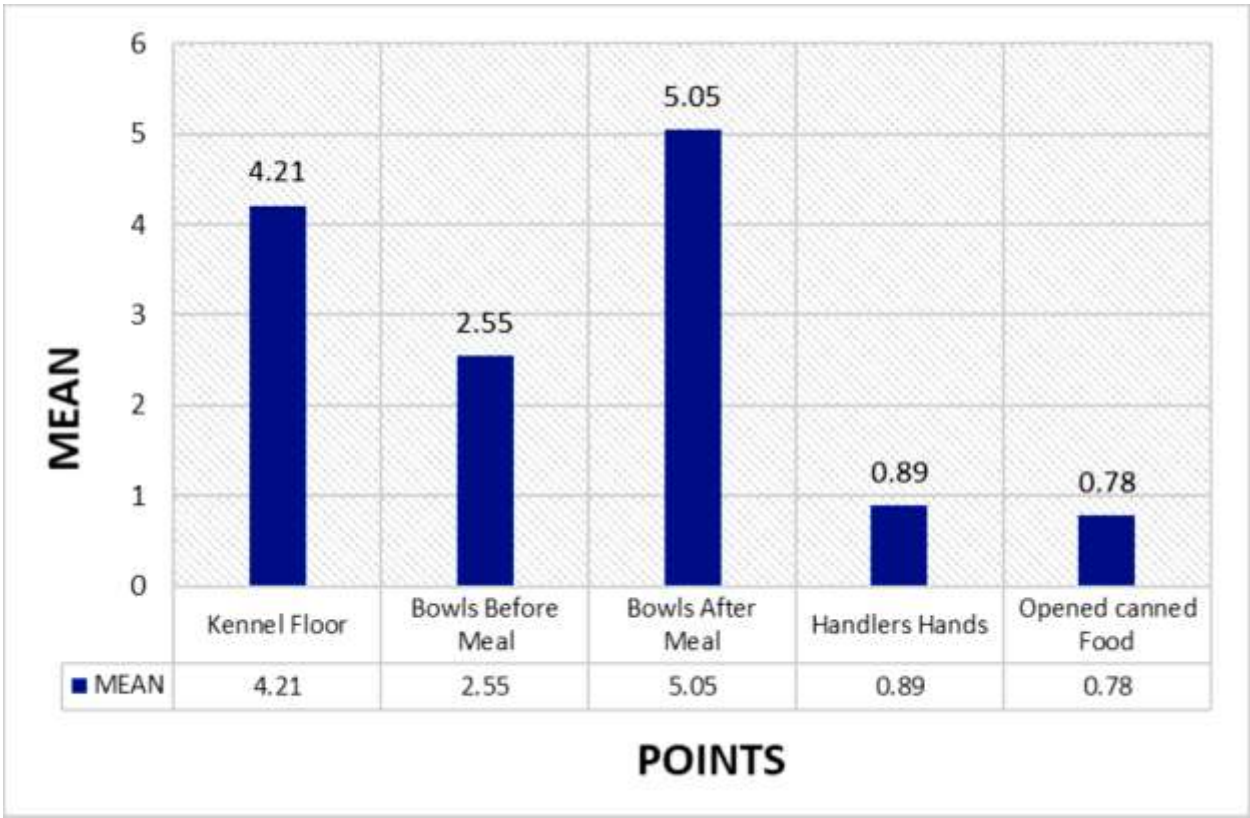


Figure (2): illustrates the Mean of Total Bacterial Contaminants Distribution at the Stages of Feeding process in AlAin police K9, (Abu Dhabi Emirate)

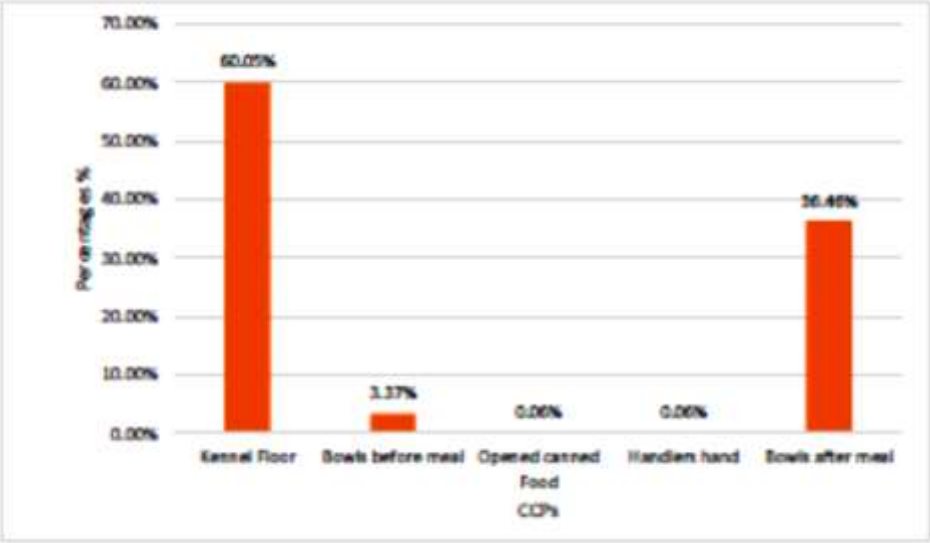
Data Analysis of Isolated and Identified Bacteria

Only two types of bacteria were isolated and identified at the five stages of feeding process, *Staphylococcus aureus*, and *Escherichia coli*.

Table (2) illustrates the concentration (%) of *E. coli* at the different stages of feeding process. High load of contamination by *E. coli* was detected at kennel floor (60.05%) and at the bowls after meal (34.46%). Neglected load was seen at the points of canned food and handlers' hands.

Table 2: Load of *E. coli* (%) at Different Stages

CCPs	Percentage %
Kennel Floor	**60.05%**
Bowls before meal	**3.37%**
Opened canned Food	**0.06%**
Handlers hand	**0.06%**
Bowls after meal	**36.46%**
Total	**100%**



High load of contamination by *Staphylococcus aureus* was detected at the bowls after meal (70.89%) and the Kennel floor with a percentage of (25.54%). No detectable *S. aureus* was seen in the canned food, and very low in handlers' hands (Table 3).

Table 3: Load of *Staphylococcus aureus* (%) at Different Stages

CCPs	Percentage %
Kennel Floor	**25.54%**
Bowls before meal	**3.23%**
Opened canned Food	**0.00%**
Handlers hand	**0.34%**
Bowls after meal	**70.89%**
Total	**100%**

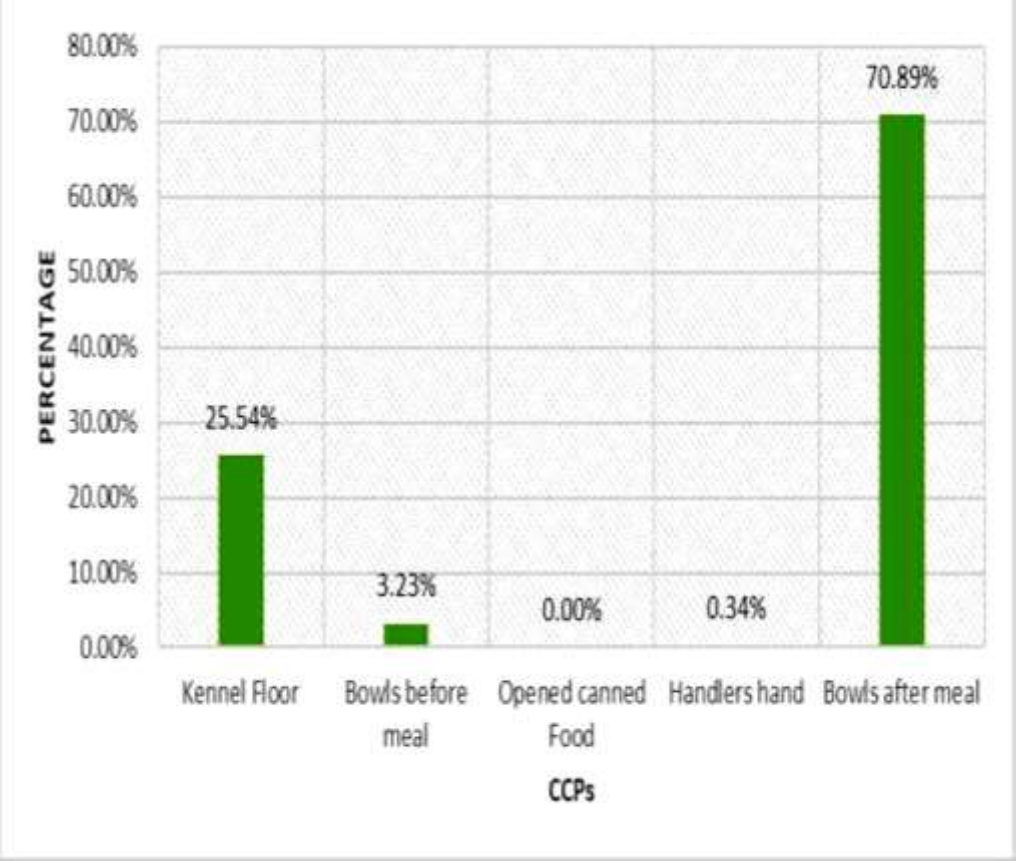


Figure (4) illustrates the concentration (%) of *Staphylococcus aureus* at the different stages of feeding process in AlAin police K9, (Abu Dhabi Emirate)

The obtained results indicate that *S. aureus* is the most contaminant bacteria than *E. coli* at the different stages. *E. coli* is mostly seen in high load at the kennel floor rather than other stages.

Table 5: **Mean and standard Deviation, of Total Viable Counts of *Staphylococcus aureus* and *E. coli* ((log^{10} cfu/cm^2) at Different Stages of Feeding Process in AlAin police K9, (Abu Dhabi Emirate)**

CCP	*S. aureus*	*E. coli*	TOTAL
Kennel Floor	24.87%	1.58%	26.45%
Bowls before meal	3.15%	0.09%	3.24%
Bowls after meal	69.03%	0.96%	69.98%
Handlers hands	0.33%	0.00%	0.33%
Opened canned food	0.00%	0.00%	0.00%
TOTAL	97.38%	2.63	100.00%

As shown in table 4 the highest contamination was seen at the bowls after meal (69.03%) by *S.aureus* and *(*0.96%) by *E.coli* .

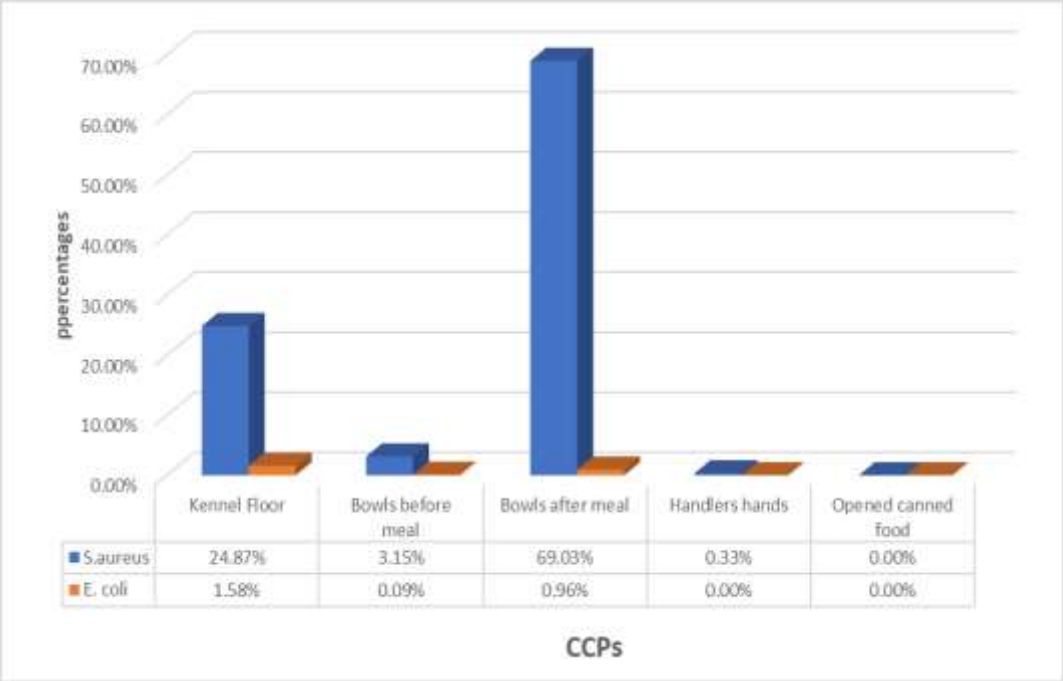


Figure (5) Level of contamination of *Staphylococcus aureus* and *Escherichia coli* isolated and identified at the different stages of dog feeding process.

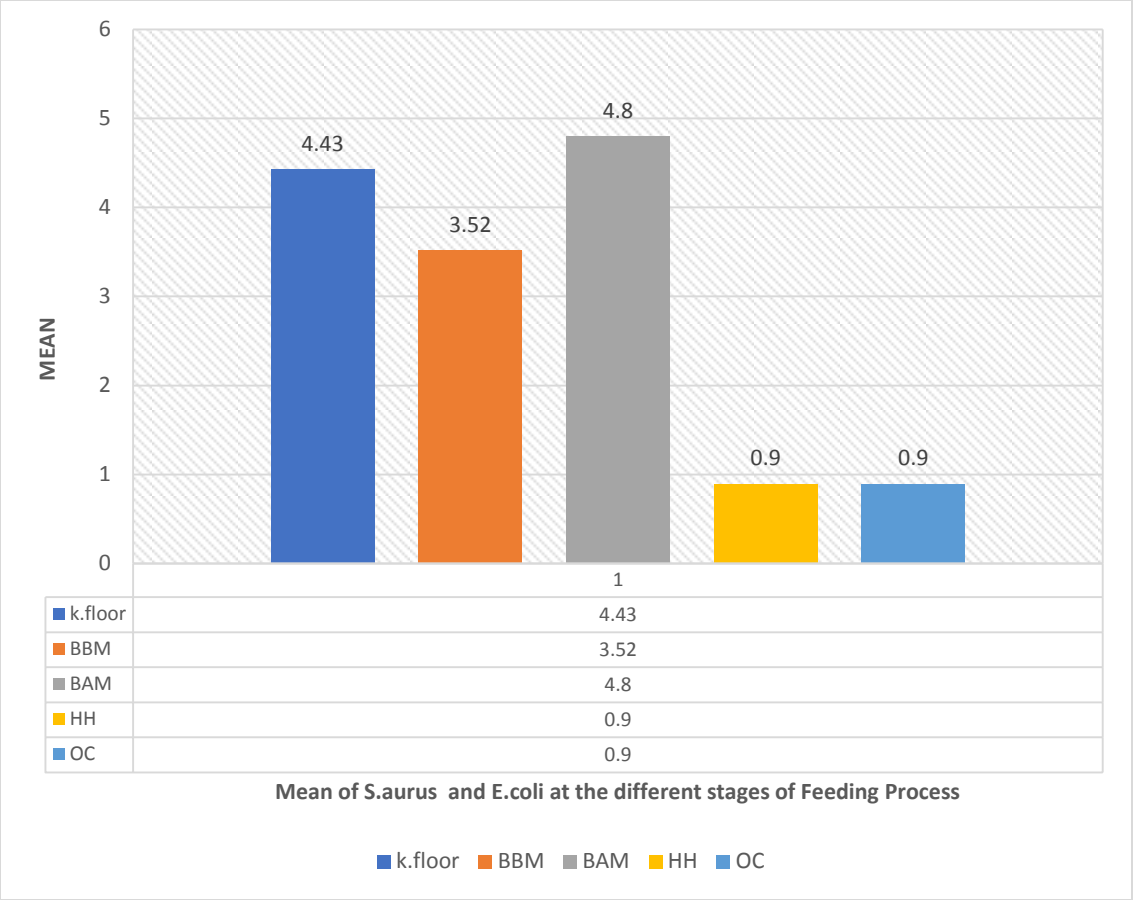


Figure (6) Mean of *Staphylococcus aureus* and *E. coli* at the different stages of dog feeding process.

The highest contamination at bowls after meal, mean: 4.8 and lowest contamination at the Handlers hands and the canned food, mean 0.90 in both stages.