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Identification of the Pathogenic Bacteria Contaminated Canine Feeding Process

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A B S T R A C T

This study was conducted in Al-Ain (Abu Dhabi Emirate) at the Police Dogs Unit K9, to detect the load of bacterial contamination at the critical control points of canine feeding process and these are 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. 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). Accordingly, *Staphylococcus aureus* and *Escherichia coli* were seen 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.

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 best food is one that meets all the nutritional requirements necessary for gog health. Dry food which is the most economical type of commercial food. But canned food or wet food has shelf live and preferred by dogs. The least nutritional value of all dogs feed is semi-moist food (Pork, burgers or other meaty food). While home cooked food used by owners is time consuming and expensive. Whereas, raw food (mixture of bones and organs) is good for

many dogs (Nylabone, 2019).Macrobiotic of canine digestive tract plays a major role in defensive mechanism (Gison and Wang, 1994). The growth of foreign microorganisms entering the tract with food and water is inhibited by the action of the antimicrobial substances of the intestinal flora and dogs are quite resistant to *Salmonella* spp. and require a large dose to cause infection (Carnivora, 2019). The aims of canning process to prevent food from spoilage and preserve the quality of the food and kept it for an extended period of time without refrigeration and without loss of nutrition values (Blumental, 1990). But microorganisms that contaminated 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. Also, environmental temperature and oxygen availability influence the bacterial growth in opened canned food (FDA, 2001). There are many types of oral bacteria in dogs also can cause severe diseases and death (Kaisompimolpore *et al.,* 2003; Dewhirst *et al.,* 2012).

Training and education of food handlers are best ways for food safety that by knowing safe cooking temperature, proper storage of high – risk food, sanitation measures, sound management and personal hygiene (AIFS, 2016). The aim of this study was to assess and identify the pathogenic bacteria and their load in the critical control points of canine feeding process.

Materials and Methods

Area study

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

Collection of samples

A total of 300 swab samples were collected from dogs' kennels, 30 swabs was 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 laboratory for Microbiological analysis.

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

Collection of swab samples for microbiological testing

Selecting sampling area of about 10 cm X 10 cm (or 20 cm x 20 cm). The swab was rubbed and rolled firmly several times across the sampling area, then the samples were labeled and preserved until used.

Total Viable Count (TVC)

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

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 was be prepared. One ml of nutrient broth with drawn 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 was 1/100and the process was repeated until reach 1/100000 concentration. 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 (count plate). The plate incubated overnight at 37^0c for 24 hours. The colonies were counted after formation (Miles and Misra (1938)

Statistical analysis

Data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16.0, SSPS Inc. And Chicago, IL, USA). All bacterial counts were converted to log $_{10}$ CFU/CM^2. All bacterial counts were analyzed, and descriptive statistical method and ANOVA were performed. Statistical significance was set at a P-value of ≤0.05 (Table 1).

Results and Discussion

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

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.

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 and 4). The study shows a statistically significance difference at (P ≤0.05) for the critical points.

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 Al-Ain _City

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 %

Table.2 Evaluation of *E. coli* (%) at Different Stages of dogs (n=250) feeding process in Al-Ain _City

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 Al-Ain _City

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%

Table.4 Percentage of *Staphylococcus aureus* and *Escherichia coli* isolated and identified at the different stages of dog (n=300) feeding process in Al-Ain _City

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%

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 (Rita *et al.*, 2007). *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. They are subject 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

that will destroy the vegetative cells of pathogenic bacteria. The prevention of recontamination following heating, then, it is the critical control step in their processing (İnal *et al.,* 2018).

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 are 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. These finding 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, 2009) 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.

The data obtained from the kennel floor show 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 are 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 this study, though, standard cleaning protocols should be implemented to minimize Coliform recovery

to promote dog physical health and hygiene and prevent cross-contamination.

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), 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 organism 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 of *S. aureus* can be y 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 can 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. In conclusion, the results clearly show that there is 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.

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