THESIS

GROUND BEEF PATHOGEN DYNAMICS AND THE CURRENT SCOPE OF THE IMPACT OF FOREIGN MATERIALS ON MEAT AND PET FOOD PRODUCTS

Submitted by

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ABSTRACT

GROUND BEEF PATHOGEN DYNAMICS AND THE CURRENT SCOPE OF THE IMPACT OF FOREIGN MATERIALS ON MEAT AND PET FOOD PRODUCTS

This thesis provides an overview of ground beef consumption and the state of microbial testing of ground beef. The study focuses on understanding dynamic growth relationships between pathogenic and non-pathogenic bacteria in ground beef and how this information might be used to predict the presence of pathogens or the onset of microbial spoilage. Additionally, this thesis examines current United States Department of Agriculture Food Safety Inspection Service (**USDA-FSIS**) and the Food and Drug Administration (**FDA**) regulations around foreign material adulterated meat and pet food. The review looks at the challenges between the two regulatory bodies and provides guidance for improvement.

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DEDICATION

To those who stand up and fight back.

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CHAPTER 1

LITERATURE REVIEW

1.1. Ground Beef Consumption

An estimated 55%- 60% of U.S. beef consumption per capita is ground, making ground beef the number one consumed beef item in the U.S. (Zimmerman, L., 2022). Ground beef is also the most significant percentage of meat or poultry class 1 recalls (Ollinger, M. et al., 2020, Painter, J. et al., 2018). USDA-FSIS reported that over 13 million pounds of ground beef were recalled between April 2017 and March 2022 due to possible *E. coli* O157:H7, *E. coli* O103, *E. coli* O26, *Salmonella* Dublin, and *Salmonella* Newport (USDA-FSIS, 2022). Of these 23 recalls, 20 were attributed to possible *E. coli* (USDA-FSIS, 2022). Recalls of any size cause consumers to lose substantial trust in the safety of the products they purchase. Research must focus on decreasing the prevalence of these pathogens in ground beef to decrease the occurrence of foodborne illnesses, the economic burden they cause, and the number of recalls associated with short shelf-life.

The inflation-adjusted economic burden of the 15 leading foodborne pathogens identified by the U.S. Centers for Disease Control and Prevention (CDC) was $17.6 billion in the United States with Salmonella, *E, coli* non-O157, and *E. coli* O157 accounting for $4.48 billion (Hoffman, S. et al., 2021, USDA-ERS, 2021). This number incorporates the costs for medical care, productivity loss, and deaths associated with each pathogen. The complexity of microbial communities of food products must be understood to decrease the economic burden caused by foodborne pathogens. Traditionally, microbial research of meat products is categorized into two

groups-pathogenic bacteria and spoilage bacteria. Understanding how these different bacteria interact as a community is imperative to reduce food safety risks and improve product shelf-life.

1.2. Pathogenic Bacteria in Ground Beef

Ground beef was the reservoir in the first-ever *E. coli* O157:H7 outbreak in 1982 and also serves as a reservoir for many other foodborne pathogens such as non-O157 Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. (Rangel, J. et al., 2005, Weinroth, M. et al, 2019). The U.S. Department of Agriculture Food Safety Inspection Service (USDA-FSIS) considers *E. coli* O157:H7 and non-O157 STECs as adulterants in ground beef products (USDA-FSIS, 2012). Unlike adulterants, which are zero-tolerance, USDA-FSIS has a maximum threshold for *Salmonella* spp. (USDA-FSIS, 2019).

1.3. Microbial Degradation on Shelf Life

The nutrient composition, pH, and moisture content of meat are highly suitable for the growth and survival of many microorganisms. Thus, the activity of microorganisms results in meat spoilage (Casaburi, A. et al., 2015). Packaging and storage temperature are the most significant factors impacting microbial degradation (Brooks, J. et al, 2008, Casaburi, A. et al., 2015). Mainly, refrigeration temperatures affect the growth of psychrotrophic bacteria, and packaging type can either be favorable for anaerobic or aerobic microbes (Casaburi, A. et al., 2015).

Spoilage in ground beef is an essential subjective measurement associated with microbial counts, color, texture, taste, and odor changes due to microbial activity (Brooks, J. et al., 2008, Gill, C., 1986). Microbial counts of 10^7 and 10^8 log CFU/cm^2 can produce off-odors and slime on meat products (Aryes, J., 1960, Brooks, J. et al., 2008). Microbes belonging to the groups of *Enterobacteriaceae*, *Brochothrix thermosphacta*, pseudomonads, some clostridia, and lactic acid

bacteria (LAB) are known pathogens associated with the meat environment that can also cause quality concerns for ground beef (Casaburi, A. et al., 2015, Weinroth, M. et al., 2019).

1.4. Pathogenic and Nonpathogenic Bacteria as a System

Ground beef's shelf-life is influenced by pathogenic and nonpathogenic bacteria and should be considered a system. Antagonistic and coordinated behavior and interactions of growth of one organism favoring the growth of another organism are some interactions observed in food systems (Gram, L. et al., 2002, Casaburi, A., et al., 2015). For example, *Enterobacteriaceae* is an indicator organism typically used to predict the presence of pathogens (Weinroth, M. et al., 2019).

Culture-independent methods are used to predict the presence of pathogens on ground beef. Predictive microbial models are often utilized to assess food safety risks. These culture-independent methods are under static environmental conditions and do not account for the microbiome as a system (Powell, 2003). Utilizing culture-independent and bacterial culture methods provides a broader, more systematic view of the microbiome.

1.5. NextGen Sequencing

1.51. History of Culture Techniques

Traditional predictive microbial models only provide monospecific culture results, and they use a static environment that does not consider the diversity of bacterial communities (Powell, M. et al., 2004). Although this type of modeling is beneficial when studying a targeted organism, it fails to account for the interspecific competition within and diversity of pathogen populations in food products that are naturally contaminated (Powell, M. et al., 2004, Weinroth, M. et al., 2019). Complex predictive methods produce uncertainty and variation within their results but combining these models with monospecific culture predictive methods to create a

system modeling approach can provide more well-rounded results that give insight into pathogen population dynamics within the microbial community (Powell, M. et al., 2004).

1.52. Modern Technologies Used in Research

High Throughput Sequencing (HTS) technologies such as Shotgun metagenomics and 16s rRNA amplicon sequencing are currently used to examine the microbial communities of meat products. 16s rRNA uses polymerase chain reactions (PCR) to amplify genetic materials before sequencing.

Whole Genome Sequencing (WGS) via HTS methodologies has become more popular with technological advancements and cost reductions (Therrien, D.A. et al., 2021). This type of sequencing relies on either short-read or long-read sequencing platforms. Compared to traditional methods, WGS provides greater resolution and selective power for differentiating closely related species (Therrien, D.A. et al., 2021). However, Therrien, et al. found that the variety of workflows for processing, sequencing platforms, and analysis of large data sets have created setbacks for applying WGS to the food industry (2021).

1.6. Previous Research

Previous studies that used a system modeling approach to look at interspecific competition produced results that suggest competitive interactions of microbial communities are highly susceptible to the initial conditions and random variation in interspecific competition coefficients and growth rates (Powell, M., et al., 2004). This sensitivity makes it difficult to use microbial community dynamics to accurately predict the risk of pathogens on food products (Powell, M. et al. 2004).

Previous studies have used microbiome research to investigate antimicrobial resistomes found in ground beef but have not observed pathogen dynamics of the ground beef microbiome.

Weinroth, M. et al. (2019) observed the effects of antimicrobial decontamination interventions and product storage on the ground beef microbiome using culture-based methods alongside 16S rRNA gene sequencing and found the combination of approaches to be highly complementary. Specifically, 16s rRNA gene sequencing provided a greater level of clarity into the microbiome leading to descriptions of lesser-reported bacteria and confirmation of dominant phyla (Weinroth, M. et al., 2019).

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CHAPTER 2
GROUND BEEF PATHOGEN DYNAMICS

Overview

Ground beef is a reservoir for both pathogenic and spoilage bacteria. Between April 2017 and March 2022, USDA-FSIS reported that over 13 million pounds of ground beef were recalled due to possible *E. coli* O157:H7, *E. coli* O103, *E. coli* O26, *Salmonella* Dublin, and *Salmonella* Newport (USDA-FSIS, 2022). Similarly, a significant volume of ground beef is lost each year due to short shelf-life primarily driven by microbial deterioration. Managing the presence of pathogenic and spoilage bacteria in ground beef products is a notable priority for the industry. As tools to measure these variables evolve, we must explore potential avenues by which we can predict the presence of a pathogen or microbial spoilage. This study aimed to evaluate changes in the microbial community of raw ground beef throughout dark storage in the presence of a pathogen.

Fresh ground beef was equally divided into two batches for evaluation as a non-inoculated control or inoculation with USDA-FSIS approved non-pathogenic *E. coli* surrogates (ATCC BAA- 1427, BAA-1428, BAA-1429, BAA-1430, BAA-1431). Before overwrap packaging, control and inoculated ground beef samples were portioned and placed on expanded polystyrene trays. Overwrapped packages were put into dark storage at 4°C. Samples were collected on days 0, 1, 2, 3, 5, and 7 for qualitative and quantitative evaluation of bacterial populations using traditional plate count methods and 16s rRNA sequencing. The project was replicated three times in a split-plot design, with replication and sample identification as random variables. Data analysis was performed using R.

As anticipated, microbial populations changed in number and composition as dark storage length progressed. Although the inoculated packages had greater numbers of bacteria, the growth trend was similar to non-inoculated controls. Unexpectedly, naturally occurring rifampicin-resistant bacteria were observed in control samples, suggesting acquiring this resistance feature from other sources. Microbial populations within each group (Control and Inoculated) were influenced mainly by inoculation but may be utilized to predict surrogate growth. As microbial technologies evolve, allowing for a more comprehensive examination of microbial populations, exploring the relationship between pathogenic and non-pathogenic bacteria is crucial. Understanding the dynamic growth relationships between these two bacterial categories may yield valuable tools to predict the presence of pathogens or the onset of microbial spoilage.

Introduction

An estimated 55- 60% of per capita beef consumption is in the ground, making it the number one consumed beef item in the U.S. (Zimmerman, L. 2022). In addition to being the most widely consumed beef product, ground beef is also a reservoir for pathogenic and spoilage bacteria. It has the greatest percentage of Class 1 recalls among meat and poultry products (Ollinger, M. et al., 2020, Weinroth, M. et al., 2019, Painter, J. et al., 2018). Between April 2017 and March 2022, USDA-FSIS reported that over 13 million pounds of ground beef were recalled due to possible *E. coli* O157:H7, *E. coli* O103, *E. coli* O26, *Salmonella* Dublin, and *Salmonella* Newport (USDA-FSIS, 2022). Of these 23 recalls, 20 were attributed to possible *E. coli* contamination (USDA-FSIS, 2022). Besides the significant public health risk posed by contaminated beef products, recalls also result in a loss of consumer trust and confidence in beef products (Kramer et al., 2005). Similarly, the economic burden of foodborne illness on

consumers and the industry is significant. The inflation-adjusted economic burden of the 15 leading foodborne pathogens identified by the U.S. Centers for Disease Control and Prevention (CDC) was $17.6 billion in the United States, with Salmonella, *E. coli* non-O157, and *E. coli* O157 accounting for $4.48 billion (Hoffman, S. et al. 2021, USDA-ERS 2021). This number incorporates the costs for medical care, productivity loss, and deaths associated with each pathogen. The complexity of microbial communities of food products must be understood to decrease the economic burden caused by foodborne pathogens.

A significant volume of ground beef is lost each year due to short shelf-life primarily driven by microbial deterioration affecting perceived quality by consumers (Cassaburi, A. et al., 2014, Issanchou, 1996). The development of microbes and bacteria that consequently release metabolites while consuming meat nutrients leads to the spoilage of meat products (Casaburi, A. et al., 2014). Although meat products are considered spoiled when culture counts reach 7 logs CFU/g, some research shows microbial counts are not always a direct reflection of spoilage (Brooks et al., 2008, Ercolini, D. et al., 2006). Spoilage bacteria and other food microflora can potentially inhibit pathogen development by competition for nutrients (Powell, M. et al., 2003).

Traditionally, microbial research of meat products has focused on exploring either pathogenic or spoilage bacteria and rarely their interaction. However, the complexity of microbial communities of meat products will help create strategies to reduce food safety risks and improve product shelf-life. As tools to measure these variables evolve, we must continue to explore potential avenues by which we can predict the presence of a pathogen or microbial spoilage. Managing the presence of pathogenic and spoilage bacteria in ground beef products is a notable priority for the industry. Research must focus on decreasing the prevalence of these pathogens in ground beef to decrease the number of products not reaching shelf-life, the

occurrence of foodborne illnesses, and the economic burden they cause. This study aimed to evaluate changes in the microbial community of raw ground beef throughout dark storage in the presence of a pathogen.

Methods and materials

Beef Procurement

Four individual replications were performed by procuring four separate lots of approximately 4535.92g of ground beef chubs from two ground beef processing facilities in the United States. All lots were obtained directly from the processing plant and transported to Colorado State University Meat Lab in Fort Collins, CO. Product for replication A and B was six days old, and the product for replication C was four days old. Ground beef chubs were held in dark storage conditions in a 4°C cooler for approximately 24 hours. Each lot of ground beef represented individual replication, with four replications total.

Inoculation Prep

USDA-FSIS approved non-pathogenic *E. coli* surrogates: ATCC BAA- 1427, BAA-1428, BAA-1429, BAA-1430, BAA-1431 (ATCC, Manassas, VA) were activated individually and repeated in duplicate. One colony was transferred to 100ml of Phosphate Buffered Saline (PBS) (Sigma-Aldrich, Inc., Saint Louis, MO) plus rifampicin and incubated for 24 hours at 35°C for each strain; this process was repeated to ensure a single genetic origin for each surrogate. After the second round of incubation was completed, the surrogates were washed thrice using a Sorvall Legend X1R Centrifuge (ThermoFisher Scientific, Waltham, MA) at 4°C at 6000xg for 15 mins as well as PBS. Next, the surrogates were combined to create the non-diluted inoculum. Serial dilutions were then completed with PBS to dilute the inoculum to 4 logs CFU/g. The inoculum was plated on Tryptic Soy Agar (TSA) (Neogen Corporation, Lansing,

MI) and TSA plus rifampicin plates to ensure the targeted inoculation concentration was obtained on the day of inoculation.

Inoculation of Ground Beef

One ground beef chub was mixed using a commercial mixer to create a single source population. After mixing, one-half of the ground beef was separated for control and coarsely ground (10mm grind plate) through a #22 Big Bite Meat Grinder (LEM Products, Chester, OH). After grinding, the ground beef, 200g aliquots of ground beef were placed on white Styrofoam foam (#2PS) trays with attached soaker pads. The sample trays were wrapped with polyvinyl chloride film and designated for the control treatment group. Wrapped control trays were randomly placed in incubators set at 4°C for temporary holding to prevent temperature abuse.

The remaining half of ground beef was weighed to approximately 1814.37g and inoculated with 10mL of non-pathogenic *E. coli* surrogate inoculum at 4 log CFU/g. Inoculation occurred by mixing the sample in an industrial KitchenAid mixer (KitchenAid, Harbor, MI) at low speed for 2 mins. The mixer was paused every 30 seconds for the sample to be pushed down off the paddle to ensure homogeneity. After inoculation, the product was coarsely ground (10mm grind plate) through a Meat Master to ensure ground beef texture. After grinding the ground beef, 200g aliquots of ground beef were placed on white Styrofoam trays with attached soaker pads. All sample trays were wrapped with polyvinyl chloride film (40- gauge, Berry AEP1504310) and designated for the inoculation treatment group. Wrapped control trays were randomly placed in incubators set at 4°C for holding. Overwrapped trays from the inoculation treatment group were randomly placed in incubators set at 4°C for temporary holding to prevent temperature abuse.

All control and inoculation group samples were randomly placed in rigid cardboard boxes to replicate dark storage conditions and stored for a maximum of 7 days at 4°C (2-6°C) (standard deviation= 3.34). Two Multitrip temperature data loggers (Temprecord International Ltd, Auckland, New Zealand) were placed within the boxes to track temperatures. After each sample day, the remaining samples in the cardboard boxes were repacked to ensure randomization during dark storage.

Microbial Evaluation of Ground Beef Samples

Samples were maintained for seven days at 4°C (2-6°C), with replication A samples taken on days 0, 3, 5, and 7. Replication B, C, and D samples were taken on days 0, 1, 2, 3, 5, and 7. Five control and inoculated packages were randomly selected for each sampling day. On each sampling day, scalpels and forceps were used within an aseptic environment to remove the tray film without touching the sample product. Random sample pieces were excised until approximately 50g of sample was obtained. Each sample was placed in sample collection bags (Whirl-Pak, Madison, WI) using a sterile spoon and tongue compressor to quantify the microorganisms. The remaining ground beef sample was aseptically placed in sterile sample collection bags (Whirl-Pak) and frozen at -80°C for later evaluation of the microbial population using 16s rRNA sequencing.

For the quantification of microbial populations, 150g of Maximum Recovery Diluent (MRD) (1:3 meat to MRD ratio) (Neogen Corporation, Lansing, MI) was added to the sample and stomached for 2 mins (IUL S.A., Barcelona, Spain). Directly after stomaching, serial dilutions were completed on TSA and TSA plus rifampicin plates. Each sample was plated in triplicate. Tryptic Soy Agar plates were incubated at 25°C for 72 hours, while TSA plus Rifampicin plates were incubated at 35°C for 24 hours. After incubation, plates were stored in

incubators at 4°C and counted within three days using a Quebec Darkfield Colony Counter (Reichert, Inc, Depew, NY).

Preparation of Samples for Microbiome Analysis

Extraction Prep. Microbiome samples were initially intended to be pulverized via blender. However, due to challenges during pulverization, sample rinsates were collected instead. To facilitate the collection of rinsates, samples were stored at -20°C for at least 24 hours before adding the rinsate medium. In a sterile environment, each sample was transferred into a filtered sample bag (Whirl-Pak). Approximately 150 mL of 0.05% Tween Phosphate Buffered Saline (PBS) (Sigma-Aldrich, Inc., Saint Louis, MO) was added to the sample bag, and samples were allowed to thaw at 4°C for approximately 3 hours and 43 minutes. Once thawed, samples were hand massaged for 2 mins before removing a 20mL aliquot. Aliquoted rinsates were stored in sterile 50mL conical tubes (add manufacturing information) and maintained at -80°C for later analysis of the ground beef microbiome.

Statistical Analysis

This project was a split-plot design with treatment (control or inoculated) serving as the main plot and storage day as the subplot. The study included three replicates. Data analysis was performed in R (version 1.3.1073). Bacteria plate counts were presented as log CFU/g and evaluated in the car, tidyverse, emmeans, multcomp, lme4, lmerTest, and readxl packages in R (version 1.3.1073). The least-square means were considered separated using a predetermined alpha of 0.05.

Results

Microbial Population Results

As anticipated, microbial populations changed in number and composition as dark storage length progressed. Even though inoculated samples had more bacteria, the growth trend was similar to the non-inoculated controls.

TSA plus Rifampicin. There was sufficient evidence of an interaction between treatment and dark storage day for total coliform counts (Table 2.1). Inoculated samples had significantly higher total coliform counts than control samples.

Table 2.1. Least-Squares Means for total coliform counts for the interaction of treatment with dark storage day (p-value < 0.005, SEM=0.195)

	Total coliform counts (log CFU/g) on dark storage day*					
Treatment	0	1	2	3	5	7
Control	1.33^{ay}	1.32^{ay}	1.31^{ay}	2.69^{by}	3.25^{by}	3.54^{cy}
Inoculated	4.04^{z}	4.09^{z}	3.99^{z}	4.09^{z}	4.06^{z}	4.01^{z}

*Within a row, least-squares means lacking a common letter a, b, or c, differ significantly. Within a column, least-squares means lacking a common letter z, or y differ significantly.
[a]p-value = 1.000, [b]p-value = 0.0001, [c]p-value < 0.0001
[z]p-value =1.000, [y]p-value < 0.0001

Naturally occurring rifampicin-resistant bacteria were unexpectedly observed in the non-inoculated control samples, suggesting the acquisition of these resistance features from other sources. One possible source is the usage of USDA-FSIS-approved non-pathogenic surrogates for in-plant inoculation studies. The naturally occurring rifampicin-resistant bacteria displayed morphologies that differed from the surrogates used for inoculation.

Discussion

As new microbial technologies evolve, it is crucial to continue exploring the relationship between pathogenic and non-pathogenic bacteria. Understanding the dynamic growth relationships between these bacteria groups may yield valuable tools for predicting the presence of pathogens or the onset of microbial spoilage in ground beef.

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CHAPTER 3

CURRENT SCOPE OF FOREIGN MATERIAL REGULATION IN MEAT PRODUCTION, RENDERING, AND FOOD MANUFACTURING: A REVIEW

Overview

Foreign materials, such as metal, rubber, plastic, and glass, are physical hazards that pose safety and quality risks when incorporated into food products. Over the past 20 years, plastic, metal, and glass have been the most common materials responsible for the United States Department of Agriculture (**USDA**) reported recalls (USDA-FSIS, 2020). Between January 1, 2000, and January 1, 2020, USDA reported 1,782 recalls of meat and poultry products. Of these, 11.56% were due to foreign material contamination (Stevens, 2021). In 2019, 27 extraneous material incidents caused approximately 15.5 million pounds of meat and poultry products to be recalled (USDA-FSIS, 2020). However, these numbers do not tell the whole story of the prevalence of foreign material contamination because they do not include products recalled by the FDA and other contaminated products sold to rendering facilities or destroyed products. The Food and Drug Administration (**FDA**) reported eight pet food recalls due to foreign material contamination between 2007 and 2019 (Fox, 2020). Recent estimates suggest that foreign materials cost the pet food industry more than $10 million annually (Caparella, 2020).

Within the Code of Federal Regulations (**CFR**), Titles 9 and 21 grant the United States Department of Agriculture- Food Safety Inspection Service (**USDA-FSIS**) and FDA as regulatory entities for food production. Specifically, USDA-FSIS is responsible for maintaining meat and poultry products regulations, while the FDA is responsible for rendering and pet food production (CFR, 2020). Within both titles of the CFR, the guidelines for foreign material

control are minimal and unclear, making it difficult for both producers and consumers to understand how they should mitigate or manage these hazards. For example, Title 9 CFR § 417 (2020) covers Hazard Analysis and Critical Control Point (**HACCP**) Systems that focus on preventing chemical, biological, and physical contamination in food but does not give an example of a physical hazard within the CFR. This review summarizes these limited regulations, their challenges, the implications of insufficient regulatory guidance and provides recommendations for improvement.

Introduction

A foreign material, also referred to as 'hards or sharps,' is any non-animal object found in meat and poultry products intended for consumption (ASK USDA 2019, FDA, 2005). Examples of foreign materials commonly found in meat and meat products include glass, metal, rubber, wood, and plastic. Products contaminated with foreign materials have the potential to cause injury, such as lacerations and choking (FDA, 2005). Although foreign materials in food are firstly a food safety and public health concern, they also pose a significant quality concern as contaminated products can lead to consumer complaints, brand mistrust, and economical losses.

The United States Food and Drug Administration (**FDA**) requires regulatory action when a product contains a foreign material 7mm to 25mm in length (FDA, 2005). Notably, foreign materials over 25 mm in length are exempt from this stipulation as it is assumed they would be identified and removed before consumption. Similarly, the United States Department of Agriculture Food Safety Inspection Service (**USDA-FSIS**), foreign materials are considered physical hazards under Hazard Analysis and Critical Control Point (**HACCP**) plans (9 CFR, 2020 § 417). Regulatory action is not required when a facility discovers a contaminated product

falls outside specifications of 7mm to 25mm in length. This leaves the manufacturer to decide whether or not they will take action, such as recalling the product due to quality concerns.

Foreign materials are a common issue within food production; however, given the concerns described above, controlling contamination prior to products entering commerce is crucial to ensuring product quality and safety (Stier, 2016). Currently, manufacturers use various methods and programs, such as Good Manufacturing Practices (**GMPs**), to prevent physical hazard contamination. In addition, many facilities utilize x-rays, sifters, metal detectors, magnets, vision systems, and sorters to detect and remove foreign materials throughout the production process (Stier, 2016). Despite these programs and equipment, foreign material contamination remains a prevalent and growing issue. Between 2000 and 2020, the United States Department of Agriculture (**USDA**) reported 1,782 recalls, of which 206 were due to foreign material contamination (Stevens, 2021). Plastic contamination was responsible for 56% of these (Stevens, 2021). In 2019, USDA reported 15,573,818 pounds of recalled products due to extraneous material contamination (USDA-FSIS, 2020).

Currently, there is no publicly available data on the total impact of foreign materials in the meat and pet food industries because manufacturers are not obligated to report contaminated products sent to rendering facilities, products destroyed before distribution, or the total volume of products impacted by recalls. However, the FDA reported eight pet food recalls due to foreign materials between 2007 and 2019 (Fox, 2020). The variation in regulation of foreign materials between USDA-FSIS and FDA may contribute to the much lower number of FDA-reported pet food recalls. Nonetheless, the 163 million dogs and cats within the United States rank 5th in global meat consumption; thus, physical contamination poses a safety, quality, and financial concern for pet food producers (Caparella, 2020, Okin, 2017).

Foreign material contamination is a unique challenge because contaminants, such as bones and rocks, can naturally occur. Employees and facilities can also introduce foreign materials such as metal, plastic, paper, and other objects to products. The complexity of the contaminants and how they enter into the production streams creates challenges in educating employees and implementing solutions focused on eliminating the presence of foreign materials. Similarly, different regulatory structures within USDA and FDA may further complicate mitigating foreign materials in meat and meat-related products.

As a result, addressing the foreign material challenge is complicated and largely dependent on clear guidelines and regulations. The purpose of this review is to summarize current federal regulatory structures related to foreign materials in meat, rendered products, and pet food. Additionally, this review will highlight the challenges and implications of variable regulatory guidance on producing safe meat and pet food products.

A Brief History Of U.S. Regulations

Origins of the Code of Federal Regulations

In the past, the United States published laws and regulations in different forms, such as bulletins and pamphlets, making it very challenging to keep track of these laws and their updates or implementation. In 1935, the Federal Register Act was passed to combat this challenge (Mckinney, 2019). This Act declared that the Federal Register would be composed of all agency rules and regulations, executive orders, and presidential proclamations in conjunction with proposed rulemaking after the Administrative Procedures Act in 1946. The Federal Register Act was amended in 1937, creating the Code of Federal Regulations (**CFR**). This codification system consists of general and permanent rules written by departments and agencies within the federal government (Mckinney, 2019, U.S. Government Publishing Office, 2021). Although the CFR

serves many purposes, one specific purpose is to define terminology for those using the document for guidance or regulatory purposes. Originally, the CFR was updated annually to reflect modifications and changes to policies and procedures, but in October 1972, it was changed to be updated quarterly. The CFR is now divided into four groups, with each agency revising titles and regulations quarterly (Mckinney, 2019).

Current Status of the Code of Federal Regulations

Currently, the CFR is publicly available and easily accessible electronically (here). In addition to the CFR, USDA-FSIS agency employees often utilize Directives to offer specific guidance on various topics that need immediate attention. Directives are often used in place of updating the CFR (FSIS Directives). The combination of the CFR and Directives provides regulators and processors administrative guidance with topic-specific information that applies to existing CFR language. If administrative policies or regulations need to be updated, a CFR update would be utilized instead of issuing a USDA-FSIS Directive. Similar to Directives, FDA utilizes Guidance for Industry (**GFI**) which can be found here.

Regulating Animals and Animal Products (USDA-FSIS): Title 9 of the Code of Federal Regulations

Within its 50 titles, the regulations for USDA-FSIS and FDA are located under Title 9 and 21, respectively. The responsibilities and regulations for USDA-FSIS, including agency organization, mandatory and voluntary inspection of meat and poultry products, administrative provisions, regulatory requirements, and more, are located in Title 9, *Animals and Animal Products Vol II Chapter III* (U.S. Government Publishing Office, 2021, CFR, 2020). Under 9 CFR § 300.2 (2020), USDA-FSIS is awarded most of its regulatory authority and is responsible for implementing numerous statutes, including the Agricultural Marketing Act of 1946 [amended

(7 U.S.C. 1621-1627)]. The Agricultural Marketing Act of 1946 includes "certified products for dogs, cats, and other carnivora," which offers guidance for the uses of animal products in pet food manufacturing (9 CFR, 2020 § 300.2). Specific guidance to those producing products intended for use in pet food production streams and regulatory agencies who determine policies for assuring the quality and safety of animal proteins intended for pet food processing are found in 9 CFR § 355 (2020).

Challenges Associated with Title 9 of the USDA-FSIS Code of Federal Regulations

Unclear Language and Definitions in Section 9 of the Code of Federal Regulations

Throughout Title 9 Chapter 3, the regulatory language utilized within the CFR is often unclear and can create challenges when implementing guidance policies in production. Specifically, USDA-FSIS uses circular language that leads readers through multiple sections without much clarity. This confusion has significant and tangible impacts on the safety of meat and meat products produced under USDA-FSIS inspection. An example of this ambiguous language is within the definitions of edible and inedible products. These terms are first defined under 9 CFR § 301.2 (2020) and are only used to reference edibility related to humans. Although other sections of the CFR correlate to the production of proteins for non-human consumption (i.e., pets and livestock), the lack of clarity in definitions presents challenges in enforcing regulations across the industry, including pet food manufacturing (CFR, 2020). In addition to the lack of encompassing definitions, the CFR also fails to offer definitions for specific products and processes—such as offal and tanking, which can be challenging for animal protein products taken outside of human food production systems (9 CFR, 2020 §314). Figure 3.1. details the multiple definitions and production streams that encompass these products. This figure highlights the definitions of "inedible" and "edible" changing throughout the production stream. These

various definitions can cause confusion about what products regulations refer to, especially when referencing animal and human food.

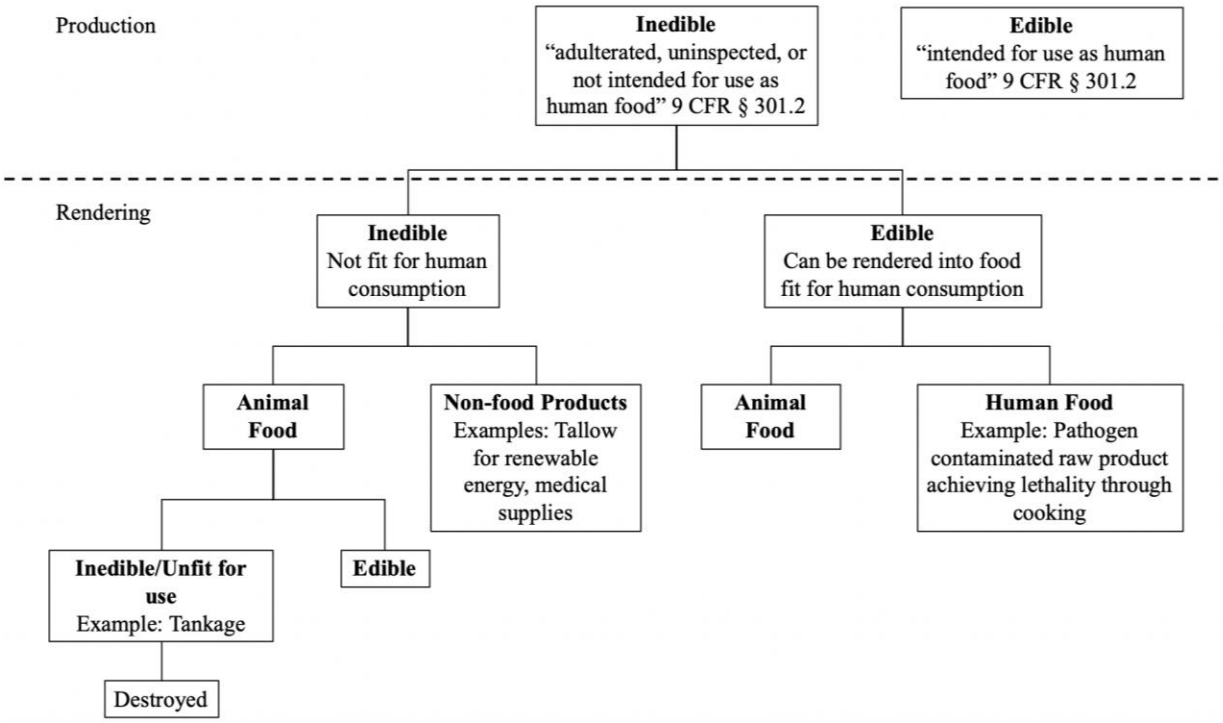

Figure 3.1. United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) Edible versus Inedible Production Systems Flow Chart
This flow chart represents the flow of inedible and edible production systems. Information provided by USDA-FSIS via the 2021 revised version of the electronic Code of Federal Regulations.

USDA-FSIS Regulations Regarding Foreign Materials

The USDA-FSIS defines adulteration as any carcass, part, or meat product if it contains any poisonous or deleterious substance that may render it injurious to health (9 CFR, 2020 § 301.2). If rendered materials are considered part of a carcass and sold, then by the CFR, rendered products containing foreign materials should also be regarded as adulterated. The extension of this regulation and definition is unclear, warranting further clarification of expectations for adulteration and rendering.

Currently, USDA-FSIS does not define foreign material or foreign matter within Title 9 Chapter 3 of the CFR. The only reference that could expand to include foreign materials is the requirement that physical hazards must be incorporated into a HACCP plan (9 CFR, 2020 § 417). While foreign materials are not explicitly referenced in this subchapter, instead, they are defined in USDA-FSIS Directive 7310.5 (2003) as "any non-animal object such as metal, rubber, glass, wood, steel and lead shot." According to this same Directive, the presence of foreign materials in a product would require that the product be deemed 'adulterated'- regardless of type, size, or quantity of the foreign materials contamination (USDA-FSIS, 2003). Even though bone is not a foreign material by definition, some still consider it to be one, as its presence can be a choking hazard. Although foreign materials are not explicitly addressed in Chapter 3 of Title 9 CFR, their declaration as a safety hazard by USDA-FSIS implies that facilities must address foreign material contamination in their HACCP plans (USDA-FSIS, 2003, CFR, 2020).

Without specifically defining foreign materials or physical hazards within the CFR, individuals must determine what physical hazards exist, including any potential foreign materials. The diversity of foreign materials found in the meat and pet food industries makes this approach inequitable for producers. The dissimilarity defining foreign materials may serve as a challenge in mitigating the impact of foreign materials.

Another practical example of confusing language that impacts foreign material presence in meat product streams is the utilization of rendered products in pet food production. Rendered or condemned products used in pet food production streams often have limited labeling requirements (i.e., just 'rendering' or 'condemned') (9 CFR § 325.11, 2020). If the CFR required rendered or condemned products intended for pet food production streams to be labeled as 'pet food,' the likelihood of foreign material contamination by an employee could be decreased.

According to 9 CFR § 325.11 (2020), animal food must be appropriately labeled as animal food or not represented for human food and have been denatured. This means animal food can be produced from products with limited labeling, i.e., just 'rendering' or 'condemned.' If the CFR regulations only allowed animal food to be prepared, sold, and transported when properly labeled for animal food, the likelihood of products adulterated with foreign materials being sold and transported could be reduced. The CFR also explains labeling requirements for animal food when it comes to selling and transporting products (9 CFR § 317, 2020). If these labeling requirements were also for product preparation, it would help create better traceback and more conscious use of rendering bins within slaughter facilities.

Another example of terminology complications is within 9 CFR § 355.31 (2020), USDA-FSIS states, "no container which bears or is to bear a label as provided under this part except with certified products which have been inspected in compliance with this part, which are sound, healthful, wholesome, otherwise fit for dogs, cats, and other carnivora, and which are strictly in accordance with the statements on the label." Within the CFR, USDA-FSIS does not explain nor define what is 'fit for dogs, cats, and other carnivora,' 'capable for use in animal food,' 'fit for animal food,' or any other similar term. If a term such as 'fit for pet food' was defined, the definition could include foreign material contamination. The term could also be used for rendered products being sold to pet food manufacturers, allowing facilities to separate these products from ones exiting the food chain, such as rendered materials going to the production of bio-diesels or pharmaceuticals.

Challenges of Foreign Material Regulation by USDA-FSIS in Products Deemed for Pet Food Production

Following the current USDA-FSIS guidance and standards applied to foreign materials and their presence in meat and meat products, any incoming product containing foreign materials is adulterated. While this status and its implications are straightforward in products intended for human consumption, a challenge arises when products containing foreign materials are diverted to pet food production. This issue has been put forward to USDA-FSIS. In response to customer complaints on adulterated products, USDA-FSIS published a notice titled *Availability of FSIS Guideline for Industry Response to Customer Complaints* (2019) and opened it for comments. While addressing comments explicitly related to foreign materials and pet food, USDA-FSIS stated, "except for the fee-for-service program for certifying products for dog and cat food in 9 CFR part 355.29, USDA-FSIS does not inspect pet food or products intended for pet food. However, FSIS revised the guideline to include language recommending that FSIS-inspected establishments communicate with pet food manufacturers before sending products to a pet food facility to ensure that the products are eligible under FDA requirements and are acceptable to the pet food manufacturer" (Kiecker, 2018).

Although USDA-FSIS does not inspect pet food, they hold regulatory oversight for animal products that will go into pet food manufacturing and offer a voluntary "Certified Pet Food" program under 9 CFR § 300.2 (2020). Specific to foreign materials, the contamination of a product with foreign materials can occur at any point along the supply chain, meaning that continuity in foreign material control is imperative to assure the safety of animal products going into pet food manufacturing streams (CFR, 2020). For this reason, cooperation and continuity in foreign material control between FDA and USDA-FSIS are critically important.

Regulating Animal Feeds and Related Products (FDA): Title 21 of the Code of Federal Regulations

The 1906 Pure Food and Drugs Act started FDA's modern regulatory functions that were later published under the Federal Register in 1935 (FDA, 2018). *Title 21: Food and Drugs Chapter 1 Subchapter E- Animal Drugs, Feeds, and Related Products* of the CFR outlines the regulatory responsibilities of the FDA as they pertain to the production of animal food (21 CFR, 2022). The majority of the regulations within this subchapter pertain to animal drugs and medicated feed for animals. Part 507 of 21 CFR *Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventative Controls for Food for Animals* specifically focuses on regulations regarding the production of pet food (21 CFR, 2022).

Challenges Associated with Title 21 of the FDA Code of Federal Regulation

Circular Language and Unclear Definitions in Section 21 of the Code of Federal Regulations

The FDA uses different terminology than USDA-FSIS to define foreign materials. Particularly, FDA uses 'hard or sharp foreign objects' to refer to foreign materials within their supporting documents but does not use or define this term within the CFR (FDA 2005). Instead, 21 CFR § 507.1 (2022) uses 'adulterated' which is when animal food violates section 361 of the Public Health Service Act and is within the meaning of section 402(a)(3) or 402(a)(4) of the Federal Food Drug and Cosmetic Act (**FD&C**). The electronic CFR provides a link to the wrong section of the Public Health Service Act (21 CFR, 2022 § 507.1). Additionally, when searching for sections 402(a)(3) and 402(a)(4) of the FD&C, the FDA further refers to chapter 21 section 342 of the United States Code (**U.S.C**) (U.S.C., 2022). These are examples of how Title 21 includes circular language that can direct the reader to different documents leading to regulatory confusion, especially when the document locations are not updated in the CFR.

Responsibility of Raw Materials and Ingredients

The FDA includes raw materials and ingredients within its definition of 'animal food' but does not specify whether this refers to incoming or already received materials (21 CFR, 2022 §507.3). The FDA does not hold any jurisdiction over the production of raw materials deemed for pet food, as USDA-FSIS is responsible for these products. Title 21 CFR § 507.12 (2020), *Applicability of this part to the holding and distribution of human food by-products for use as animal food,* states that all human food facilities that distribute by-products for animal food must comply with FDA regulations and requirements on food safety. However, meat by-products produced by USDA-FSIS, not FDA, regulated facilities are currently being sold for pet food production.

The FDA also uses the term 'supply-chain-applied control' for any ingredient or raw material that has been controlled for a hazard before it has been received (21 CFR, 2020 § 507.3). This term has the potential to be beneficial for pet food production facilities as it allows raw material and ingredient facilities to label products going to animal food that has received a corrective action for foreign material contamination as 'supply-chain-applied control products.' Having the opportunity to buy raw materials from a supplier that has applied controls for foreign materials before sale or transportation could drastically reduce the number of contaminated products the pet food industry receives.

FDA Regulations Regarding Foreign Materials

There are various routes in which the FDA oversees the production of safe animal foods including traditional recall mechanisms as well as preventative risk-based controls. In the case of foreign materials, the FDA specifically addresses foreign materials through the *CPG Sec. 555.425 Foods, Adulteration Involving Hard or Sharp Foreign Objects* (2005) and GFIs.

Unlike USDA-FSIS, the FDA provides clear definitions regarding the size of foreign material contamination, such as 7mm to 25mm (FDA, 2005). According to this document, contaminated products that fall within these sizes and are ready-to-eat or require minimal preparation that will not eliminate the hazard before consumption are considered to be adulterated (FDA, 2005).

Adding to the web of regulations is the Association of American Feed Control Officials (**AAFCO**) which FDA MOU 225-07-7001 (2019) has made responsible for providing definitions for animal ingredients and their purposes through the AAFCO Ingredient Definition Request Process. AAFCO is a voluntary association of feed control officials that focuses on consumer protection and safeguarding both animal and human health (FDA, 2020). While they do not have direct regulatory authority over pet food or human food production, they provide definitions and suggestions on protocols to FDA (FDA, 2020).

Challenges of Foreign Material Regulation by FDA Receiving Products for Pet Food

Implications of Insufficient Regulatory Guidance

Regulatory guidance in the pet food industry is quite complex. USDA-FSIS is responsible for overseeing the production of meat products, but when those meat products are entering rendering, the regulating government body is the FDA. Although the FDA is responsible for pet food production, most regulations and guidelines for hard or sharps contaminated products are originally for human food and later adapted for animal food. For example, *CPG. Sec. 555.435 Foods, Adulteration Involving Hard or Sharp Foreign Objects* (2005) was first issued in 1999 but later updated to include animal products in 2005.

Guidance for Industry #235: Current Good Manufacturing Practice (**CGMP**) *Requirements for Food for Animals* states to comply with CGMPs, it is prohibited to deliver or introduce adulterated animal food, defined under section 301(a) of the FD&C Act, into

commerce (U.S. Department of Health and Human Services, et al., 2017). Despite this, FDA does not have authority over raw materials for pet food produced under the USDA-FSIS authority. This disconnect between the FDA and USDA-FSIS creates a significant regulatory gap that production facilities are left to bridge.

Opportunities for Improvement

Manufacturing facilities, including those that produce raw meat materials, should continue to voice their concerns with FDA and USDA-FSIS to improve regulation language while also encouraging both agencies to work together to clarify agency responsibilities and regulations when the product is being transferred between facilities and agencies. Processors can continue to work with trade organizations such as the North American Renderers Association (**NARA**) which represents the interests and concerns of the North American rendering industry to the public, pet food industry organizations, governmental entities, and regulatory agencies (NARA, 2022). Most importantly, facilities should continue to work internally on education and processes focused on preventing foreign material contamination. Examples of preventative processes can be tool and gasket accountability, the use of pallet socks, glass and brittle plastic tracking, and the creation of a foreign material prevention-focused team.

Pet food manufacturers should create a supplier list to collect data on received product contamination to track which suppliers have reoccurring issues. These data can be used to create an internal scoring system to help the production team decide which facilities to purchase raw materials from. Furthermore, pet food manufacturers can share this information with the supplying facilities and work with them on decreasing the number of foreign material contamination incidences within their products.

Further advancement in technology used to detect foreign materials is critical for decreasing the number of incidences. Traditional approaches i.e., x-ray and metal detection systems, are being enhanced by innovative, accessible, and novel companies. Additional collaborative research for the application of these technologies is needed.

Educational opportunities

Facilities that sell raw materials for the production of pet food should educate their employees on what foreign materials are, the impact they have, and how to properly discard potential contamination on the production line. Collaborative efforts from processors and trade organizations to create educational materials and training tools to be used across the industry would be most impactful.

The majority of meat facilities discard foreign material-contaminated products found during production by placing them in condemned bins. These bins are sent to rendering facilities, some of which are for pet food production. Thus, making sure employees understand that the contents of condemned bins are going to pet food production and not trash will help decrease the number of contamination incidences. Furthermore, the addition of bins labeled for pet food is a great way to separate rendered products being sold for the production of pet food and for employees to visualize the difference between condemned products and products for food production.

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