Director's Digest
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PREVALENCE OF SELECTED FOODBORNE PATHOGENS IN FINAL RENDERED PRODUCTS: PILOT STUDY

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Introduction:

There has been national and international concern about foodborne illness and the safety of both animal and human foods of animal origin. Food and feed safety has become a number one priority and has stimulated animal commodity organizations, meat processors, animal feed manufacturers, animal feed ingredient suppliers and animal health care providers to establish biosecurity programs with much greater intensity. Programs designated as Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) are now common place among the respective segments of the meat industry. Indeed the rendering industry has widely formulated and implemented HACCP based programs as an element of quality assurance in fostering sanitation and hygiene in the production of rendered animal products.[(1)]

Rendering is a time/temperature process that has served as the most acceptable procedure for the safe and environmental handling of non-edible tissue ancillary to animal production and processing. Studies have demonstrated that foodborne bacterial organisms are common inhabitants in all meat producing species irrespective of general health status. As an example, E. coli 0157:H7 shedding has been demonstrated in up to 80% of individual feedlot cattle during the weekly monitoring of feedyards by the University of Nebraska. The organism was recovered at least once from each animal within a group and detected from at least one animal of the group every week of the 19-week study.[(2)(3)]

In addition to the E. coli organism, food safety concerns have focused on Listeria monocytogenes, Campylobacter jejuni, Clostridium perifringens and Salmonella spp as primary contaminants. Salmonella prevalence rates of 32% to 93% have been demonstrated in the cecal-colon contents of clinically healthy market dairy cows. [4] A pilot study at 17 rendering facilities in the Midwest United States focused on the mircrobiological examination of three specimen types from each facility for the above referenced organisms. The specimen types examined from each facility were: raw material derived from animal production and processing, material resulting from the cooking/expelling process (referred to as Crax), and the final rendered product (FRP) following the cooling, grinding, storage and transport loading.

Materials and Methods:
Seventeen commercial rendering facilities in 7 Midwestern states were visited both during winter (January-February) and summer (July-August). The facilities were randomly selected to include an industry representation of raw material, species and processing. The facilities provided 21 processing lines each of which were included in the sampling process. Two samples of each of three specimen types were obtained from each of the lines during each visit resulting in a total of 84 samples of each specimen type. All samples were appropriately labeled, placed in transport media and maintained on ice for transport to the laboratory within 30 hours. All samples were submitted to microbiological, water activity, ph and proximate analyses.

Microbiological Procedures:
After samples were logged into the receiving laboratory, they were distributed for microbiological analyses to two laboratories. Salmonella and E. coli and total plate count determinations were done in the Production Medicine Laboratory and Campylobacter jejuni, Listeria monocytogenes and Clostridium perfringens isolations were accomplished in the Laboratory for Veterinary Diagnostic Medicine, both are at the University of Illinois at Urbana-Champaign.

- ***Total Plate Counts:*** Total plate counts were accomplished using the Sim Plate™ commercial method for estimating most probable numbers (MPN).

- ***Escherichia coli*** count: *Escherichia coli* counts were done using the commercial Sim Plate™ process.

- ***Salmonella spp*** isolation and identification: *Salmonella spp* were isolated following tetrathionate enrichment using established procedures (FDA Bacteriological Analytical Manual, 8th Ed. Revision A, 1998; AOAC International, Gaithersburg, MD 20877) with modification. Briefly, because our samples were finely ground, we did not surfactant or steam treatment. Suspected *Salmonella* isolates were identified be serotyping at the National Veterinary Services Laboratory, USDA. Ames, Iowa.

- ***Clostridium perfringens, Listeria monocytogenes and Campylobacter jejuni:*** Twenty-five gram aliquots of the sample were places in sterile screw cap jars containing 80 ml of sterile phosphate buffered saline and pre-incubated for 24 hours

at room temperature. Culture procedures for each were as described (Manual of Clinical Microbiology, 6th Ed., 1995; ASM Press, Washington, D.C.).

Analytical Procedures

- **Water Activity.** Each sample of final rendered product was measured in triplicate using an Aqualab Series 3 [d] water activity meter and the mean was used as the result. Following a set of three samples, the meter was standardized using standards obtained from the manufacturer.[d]

- **pH.** Each sample was measured triplicate using the equipment (pH meter and multi-range electrodes) in the Soels Testing Laboratory and the University of Illinois at Urbana-Champaign. The mean value was used as the result. Standards were those routinely used in the laboratory. The standardization of the pH meter was examined and corrected if necessary. Following each batch of 3 samples (nine measures) standardization of the pH meter was corrected if necessary.

- **Proximate Analysis.** Proximate analyses were done commercially at Dairyland Labs, 217 East Main Street, Arcadia, WI, 54612, using "wet" chemistry processes.

MICROBIOLOGICAL RESULTS AND DISCUSSION

Raw Material:

The raw material derived from food animal production and processing for rendering was found to be highly contaminated with the five index potential foodborne pathogens that were targeted for identification. The results of the microbiological culture of the raw material are provided in the following Table No. 1.

Table 1. *Microbiological isolations of index foodborne pathogens from raw material at 17 Midwestern rendering establishments during 2 periods of sampling: winter and summer (Number of isolates/number of samples including replicates)**

Organism	Winter	Summer	Total	%
Clostridium perfringens	30/42	30/42	60/84	71.4%
Listeria spp	33/42	31/42	64/84	76.2%
1) Listeria monocytogenes	4/42	3/42	7/84	8.3%
Camplobacter spp	19/42	6/42	25/84	29.8%
1) Campylobacter jejuni	15/42	2/42	17/84	20.0%
Salmonella spp	37/42	34/42	71/84	84/5%
*Coliform spp***	42/42	42/42	84/84	100%

* Some establishments operated more than one rendering line. Sampling occurred twice at each visit for each mine, hence the denominator is based on 21 lines sampled twice each visit = 42.

** Characterization of the bacterial organisms and aerobic plate counts were not possible due to the nature of the raw material.

The heavy contamination rate for all bacterial genera/species was expected because of the nature of the material. There are numerous published surveys illustrating their ubiquist association with animal production. Clinically normal animals, harbor microbial organisms especially within their digestive systems. The presence of digestive tissue and

contents, other contaminated tissue from processing and fallen animals all contribute to raw material with high microbiological content. The raw material processed by the rendering industry thus is known to contain significant numbers of potential foodborne pathogens including a large number of Salmonella serovars. The microbiological isolations in this study verified that. Coliform cultures and the specific bacterial isolations were considered not possible due to the heavy bacterial load in the unprocessed animal raw material.

The tremendous microbial populations demonstrated to be present in the material generated by the production and processing of animals for meat serves to illustrate the tremendous environmental and biosecurity challenges presented via the antiquated alternatives of burial, landfilling, burning, composting and even on farm or central incineration should rendering not occur.

There were thirty-two different Salmonella serovars isolated from the raw material as referenced in Appendix Table No. 1 and Appendix Table No. 2.

Crax:
Isolation was not made of the index pathogens from the crax samples following the time/temperature processing used in the facilities comprising this pilot study. We believe the rendering process inactivated the target organisms. The following Table No. 2 summarizes the microbiological testing for the index foodbore pathogens in the crax.

TABLE 2. *Microbiological isolations of index foodborne pathogens from crax at 17 Midwestern rendering establishments during winter and summer.*

Organism	**Winter**	**Summer**	**Total**	**%**
*a Clostridium perfringens**	0/42	0/42	0/84	0%
b Listeria spp	0/42	0/42	0/84	0%
c Listeria monocytogenes	0/42	0/42	0/84	0%
d Camplobacter spp	0/42	0/42	0/84	0%
e Campylobacter jejuni	0/42	0/42	0/84	0%
*f Salmonella spp***	0/42	0/42	0/84	0%
*g Coliform spp***	0/42	2/42	2/84	2.4%

* Other organisms were isolated and recorded as laboratory observations but not identified within the scope of this pilot study.
** Please reference discussion.

The study had as its objective to utilize Clostridium perfringes as the index species for the Clostridium group. Clostridium perfringes is the most referenced as a foodborne contaminate as well as being a reference bacteria in some regulatory programs. Though Clostridium perfringes was not isolated from the crax material other Clostridium spp were observed as a laboratory observation. These organisms were not identified nor were they preserved for future analysis by the laboratory. A follow up study is planned for further microbiological testing for the presence of possible thermophilic species, specific identification of the Clostridium organisms and pathogenicity.

As referenced in Table No. 2 all initial Salmonella culturing were negative from the crax sampling. Reculturing the samples collected during winter to generate the data as presented above, following their storage in sterile phosphate buffered saline (PBS) for several months, yielded the unexpected recovery of Salmonella oranienburg in 8 samples from 7 separate rendering facilities. S. oranienburg was not isolated from any of the summer samples. Thus this finding warrants further study to define the origin and significance following an exhaustive laboratory and sampling quality control review during this initial pilot study. Several possibilities exist to include the thermophilic properties specific to S. oranienburg; its persistence as a contaminant on or near the discharge system (auger) of the expeller at site of sample acquisition or other quality control concerns. It is interesting to note that S. oranienburg was isolated from samples with moisture levels that were significantly lower than samples which no Salmonella spp were isolated. It was also interesting to note that S. oranienburg was not isolated from the raw material samples and of low incidence in the final rendered products.

E. coli was isolated in two of the samples obtained during the summer but winter samples were all found to be negative. This finding is also without a definite explanation due to the comparative thermal death time for the spore forming bacteria of Clostridium perfringenes which is more heat resistant than other vegetative species such as Escherichia, Listeria or Camplobacter.[5]

Final Rendered Products (FRP):
Following the actual cooking process of rendering, final product transfer and storage are accomplished under a variety of methods. As with the storage and conveying systems for all ingredients, both plant and animal, storage recontamination is possible. The following Table #3 illustrates the isolations from the final rendered products

TABLE 3. *Micorbiological isolations of index foodborne pathogens from final rendered product at 17 Midwestern rendering establishments during winter and summer.*

Organism	Winter	Summer	Total	%
a)Clostridium spp	0/42	0/42	0/84	0%
b) Clostridium perfringens	0/42	0/42	0/84	0%
c) Listeria spp	2/42	0/42	2/84	2.4%
d) Listeria monocytogenes	0/42	0/42	0/84	0%
e) Camplobacter spp	0/42	0/42	0/84	0%
f) Campylobacter jejuni	0/42	0/42	0/84	0%
g) Salmonella spp	9/42	13/42	22/84	26.1%
h) E. coli	8/42	2/42	10/84	11.9%

Clostridium perfringens, Clostridium spp, Listeria monocytogenes or Campylobacter jejuni were not isolated from any samples of final rendered products. Salmonella spp were isolated from 26.1% of total samples and E. coli from 11.9%. The Salmonella isolations were somewhat higher than those reported by the most recent American Proteins Producers Industry (APPI) but very representative for several past surveys involving both plant and animal protein sources.

Summary:
Animal production is associated with approximately 47 to 52 billion pounds of tissue not utilized as meat for human consumption. This study illustrates that this material contains a significant number of bacterial organisms to include those referenced as being specifically important to food safety and environmental biosecurity. This study verifies the highly effective process of rendering in inactivating these potential foodborne pathogens. Further studies are underway to expand upon this pilot project to continue the improvement in the quality control processes of rendering. The study provides background data that the alternatives that are often cited for the rendering process could present numerous biosecurity concerns if widely adopted.

APPENDIX TABLE NO. 1 *The Distribution of Salmonella serovars Among Raw Material, CRAX, and Final Rendered Product (FRP) During Winter and Summer Sampling Periods at 17 Midwestern Rendering Establishments.*

Winter			**Summer**		
RAW	CRAX	FRP	RAW	CRAX	FRP
Agona	Oranienburg	Oranienburg	Adelaide		Adelaide
Brandenburg		Thomasville	Agona		Brandenburg
Derby		Uganda	Berta		Infantis Illinois
Heidelberg		(Multiple)	Bovis-Morbiticans		Johannesburg
Infantis			Derby		Putten
Kentucky			Heidelberg		Senftenberg
Litchfield			Infantis		Worthington
Manhattan			Johannesburg		3, 19: Poorly Motile
Mbandaka			Kentucky		
Montevideo			London		
Muenchen			Minnesota		
Muenster			Muenster		
New Brunswick			Newport		
Newington			Ohio		
Newport			Orion		
Reading			Sentenberg		
St. Paul			Typhimurium		
Senftenberg			Typhimurium (Copenhagen)		
Tennessee			Uganda		
Thompson					
Typhimurium (Copenhagen)					
Worthington					

APPENDIX TABLE NO. 2 *Salmonella serovars Isolated from Raw Material, CRAX and Final Rendered Products at 17 Midwestern Rendering Establishments.*

Raw Material	CRAX	Final Rendered Products
S. Adelaide	S. Oranienberg	S. Adelaide
S. Agona	S. (Multiples)	S. Brandenburg
S. Berta		S. Illinois
S. Bovis-Morbificans		S. Infantis
S. Brandenburg		S. Johannesburg
S. Derby		S. Oranienburg
S. Heidelberg		S. Putten
S. Infantis		S. Senftenberg
S. Johannesberg		S. Thomasville
S. Kentucky		S. Uganda
S. Litchfield		S. Worthington
S. London		S. 3,19: Poorly Motile
S. Manhattan		
S. Mbandaka		
S. Minnesota		
S. Montevideo		
S. Muenchen		
S. Muenster		
S. New Brunswick		
S. Newington		
S. Newport		
S. Ohio		
S. Orion		
S. Reading		
S. St. Paul		
S. Senftenberg		
S. Tennessee		
S. Thompson		
S. Typhimurium		
S. Typhimurium (Copenhagen)		
S. Uganda		
S. Worthington		

References:

(1) American Protein Producers Industry, RR#2 Box 214C, Huntsville, MO 65259.

(2) Longitudinal Patterns of Fecal Shedding of Escherichia coli 0157:H7 by Feedlot Cattle – Dr. Terry Klopfenstein at al 2002 Nebraska Beef Report, University of Nebraska-Lincoln pp 29-31.

(3) Incidence of Escherichia coli in Feedlot Cattle and feedyards, 2001 Beef Report, pp 81-84 Elder et. al. 2000. Proc Nalt Acad Sci USA, p 2999-3003.

(4) Troutt, H.F., Galland, J.C., Osburn, B.I., Brewer, R.L. Technical Report: Prevalence of Salmonella in Cull Dairy Cattle at Five Slaughter Establishments Widely Separated Across the United States. 1997. FSIS-USDA pp 25.

(5) Thermal Death Time Values for Rendered Products, Dr. Annel Greene, Clemson University – FPRF Report 99A-3 January 2001.

ABSTRACT

Prevalence of Selected Foodborne Pathogens in Final Rendered Products: Piltot Study

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Seventeen rendering facilities in seven Midwestern states were visited during both winter and summer, and a total of 21 processing lines were sampled twice per visit. Samples for microbiological determination of four foodborne pathogens – *Clostridium perfringens, Listeria monocytogenes, Campylobacter jejuni* and *Salmonella spp.* – were obtained specifically from raw material entering the cooker, crax as expelled from the cooker and from stored, or to-be stored, final rendered product. *Clostridium perfringens, Salmonella spp.*, and *Campylobacter jejuni* were routinely isolated from raw material. *Listeria monocytogenes* was isolated from approximately 8 percent of the samples. Investigators judged the raw material as essentially a microbiological "soup".

Clostridium perfringens, Listeria monocytogenes and *Campylobacter jejuni* were not isolated from any of the "crax" or final rendered product samples. Additionally, *Salmonella spp.* was not isolated from any of the "crax" sampled, but were identified in 9/42 (21%) winter samples and in 13/42 (31%) summer samples from final rendered products.

Proximate analysis of final rendered products (excluding blood meal) indicated that mean values for % moisture, % crude protein and % ash were greater during the summer sampling period, but mean value for % crude fiber and % crude fat were greater during the winter sampling period. Overall, mean values for water activity and pH were slightly greater for winter samples than for summer samples of final rendered products.