Introduction
As CVM began to see an increase in the number of jerky pet treat related consumer complaints, the newly formed Vet-LIRN network (see “About the Veterinary Laboratory Response Network” background section) began to work with CVM’s Office of Surveillance and Compliance to investigate potential causes of these illnesses.\footnote{http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm} Because jerky samples from adverse event cases may come from already-opened bags containing only a few leftover pieces, we decided to approach the problem using 3 sets of product samples:

- Store-bought samples purchased by Vet-LIRN laboratories around the country
- Historical case samples collected before 2011
- Case samples collected from 2011-2013

Additional testing on animal diagnostic samples began in 2012, and is still ongoing.

We conducted bacterial, compositional and toxicological analyses, and evaluated irradiation effects on glycerin; however, each sample did not necessarily undergo all four analyses. The specific types of tests we conducted on jerky treat products include:

- **Microbiological Testing**
  - Bacterial culture for Salmonella
  - Bacterial enterotoxin
  - Mold and yeast culture
  - Mycotoxin

- **Compositional Testing**
  - Physical characteristics evaluation
  - Composition with glycerol content
  - Vitamin D content
  - DNA analysis

- **Chemical Toxicology Testing**
  - General screens for toxic compounds: restricted list
  - Metals: various heavy metals
  - Glycols: diethylene glycol-DEG; ethylene glycol-EG; propylene glycol-PEG; dihydroxyacetone (DHA); and 1,3 Propanediol
  - Glycerin metabolites: glycolic acid, diglycolic acid, and lactic acid
  - Sugar alcohols: xylitol (added in 2013), sorbitol (added in 2013), glycerol
  - Other organics: hexachlorobutadiene, paraquat, aristolochic acid, and oxalic acid (added in 2013)
  - Antibiotics: gentamicin, tetracycline degradation products, sulfonamides (sulfadiazine, sulfathiazole), trimethoprim, enrofloxacin, tilmicosin; several samples also tested for: sulfamethoxazole, sulfamethazine, sulfadiazine, sulfadiazine, sulfanilamide, sulfadimethoxine, sulfasoxazole, chloramphenicol, and gatifloxacin (both added in 2013)
  - Drugs: monensin, quinocetone, and additional forensic drug screen (list-restricted information)
  - Biogenic amines: putrescine, cadaverine, histamine, agmatine, spermidine, and spermine
Phorbol esters: *Jatropha curcas* toxins  
Additives/preservatives: nitrites, sulfites  
Tanning agents: tannic acid and gallic acid  
Flavoring agents: monosodium glutamate-MSG (added in 2013), malic acid, maleic acid, methyl-4-pentenoate (added in 2013), and fumaric acid (added in 2013)  
Illegal dye agents: Auramine, Bixin, Butter Yellow, Fast Garnet, Metanil Yellow, Orange II, Orange Oil SS, Para Red, Rhodamine B, Sudan Black B, Sudan Black I-IV G, Sudan Orange, Sudan Red 7B, Sudan Red B, Sudan Red G and Toluidine Red

**Evaluation of Jerky Treat Irradiation**  
- Furan analysis  
- 2-dodecylcyclobutanone (2-DCB)

**Vet-LIRN Diagnostic Test Rationales and Results**

**I. Microbiological Testing**

**A. Bacterial Culture for Salmonella**

The first assignment for our Vet-LIRN laboratories was the evaluation of closed-bag, store-bought samples. The laboratories each purchased 4 different jerky treat products and tested them for *Salmonella* (total of 64 samples). The remaining jerky samples were then sent to the CVM Vet-LIRN laboratory for further testing.

1. **Rationale:** Various pet foods or pet treats, such as pig ears and jerky treats (USFDA b, c), have previously been sources of human and pet *Salmonella* infections (Behravesh, et al., 2010). In 2005, FDA found 2 *Salmonella*-positive samples. *Salmonella* can cause gastrointestinal (GI) signs similar to those seen in the jerky cases reporting GI illness.

2. **Results:** All 64 store-bought samples were negative for *Salmonella*. In 2013, there were 2 recalls involving Kasel jerky treats. The first recall occurred after routine sampling by the FDA (FDA a). The second recall occurred in February 2013, after a follow-up inspection. FDA received a small number of complaints of illness in dogs who were exposed to the treats, but no reports of human illness. Kasel products are made in the US and are distributed nationwide (FDA b and c).

**B. Bacterial Enterotoxins**

1. **Rationale:** Several bacterial enterotoxins can cause hemodynamic vascular collapse and gastrointestinal changes culminating in multiple organ failure (Kocandrle et al., 1966, Taylor et al. 1982; Weese et al., 2001). Most jerky treats are a very hard and most of the products have been irradiated, reducing the chance of bacterial growth after packaging. If bacteria produced toxins prior to irradiation, however, these could potentially be present in the final product. Heating while drying the jerky may destroy the toxins; however, we asked two collaborating laboratories to test whether *Clostridium perfringens* enterotoxin and Staphylococcal enterotoxin can be found in a subset of the store-bought or historical samples. No validated methods for detecting these toxins in this type of product exist; thus, these studies are very preliminary in nature.

2. **Results:** Lab 2 tested 5 samples for the presence of *C. perfringens* enterotoxin. All samples were negative. Lab 7 tested 26 samples for the presence of *S. aureus* enterotoxin. All samples were negative.
2013 plans include culturing for *Bacillus cereus*, *S. aureus*, and *C. perfringens*, and testing for a number of related enterotoxins for *B. cereus* diarrheal toxin, *S. aureus* enterotoxin, *C. perfringens* enterotoxin and Shiga toxin. We received results for 60 samples submitted for culturing. Two samples were positive for *B. cereus*; all other samples were negative for all other microorganisms tested. Detection methods for enterotoxins are in the validation phase.

### C. Mold and Yeast Culture
1. **Rationale:** We submitted the same 5 samples to two different labs for mold and yeast culturing.

2. **Results:** Lab 3 tested 5 samples. Four samples were negative and one was positive for *Penicillium* sp.

   Lab 14 isolated *Cladosporium* sp. from 4 samples, *Alternaria* sp. from two samples, *Aspergillus* sp. from one sample, and *Penicillium* sp. from one sample.

### D. Mycotoxin Testing
1. **Rationale:** A number of consumer complaints mentioned that the jerky looked moldy. Molds can produce a variety of toxicants which have been reported to cause both renal and gastrointestinal disturbances in humans and domestic animals (Newman et al., 2007; Hayes and Williams 1978; Armbrrecht et al., 1971; Bingham et al., 2004; Harper, 2009; Tor et al., 2006). All samples were tested for mycotoxins by both labs.

2. **Results:** Three Vet-LIRN labs tested 22 samples for various mycotoxins (Aflatoxin B1, Ochratoxin, DON, Penitrem A, Roquefortine, DAS, T-2, Fumonisin-B1, and Zearalenone). All samples were negative for mycotoxins.

### II. Compositional Testing

#### A. Physical Characteristics
1. **Rationale:** Physical characteristics of the jerky (poor digestibility, extraneous fibrous material) could account for the gastrointestinal disturbances associated with their consumption, especially in small-breed dogs.

   Physical characteristics were evaluated for 20 samples and for an organic jerky product manufactured in the US. The jerky samples were soaked in phosphate buffered saline (pH 3, 5, 7) and evaluated at multiple time periods up to 44 hours. Our goal was to see if the samples were composed of solid or shredded muscle, how much tendon (if any) was present, and to look for any unusual physical appearance. The samples were photographed to document their appearance. Samples were also preserved in formalin and their histopathology examined to identify any morphologic abnormalities.

2. **Results:** We found that most of the samples remained very hard or rubbery for the duration of the experiment. Samples were placed in a Seward Stomacher 400, and 100 mL of phosphate buffered saline was added into each bag. Samples were smashed at 200 rpm for 90 minutes using an instrument called a stomacher. The jerky remained mostly intact. Similar results were observed by a different lab after jerky was rehydrated in buffer overnight and stomached the following morning. This could indicate that some of the dogs’ digestive disturbances are caused by the tough nature of the dried product. Dogs do not
chew their food; rather, they gulp whole jerky treats or minimally chew them before swallowing. Histopathologic examination revealed many jerky strips have basophilic amorphous material (BAM) located at the surface and sometimes within the strip. The nature of this material is currently unknown and will be further investigated. A few jerky samples had areas of necrosis and inflammatory infiltrate. In general, muscle could be readily recognized; striations were present in these dried jerky treat samples. Some treats were made from composite material.

B. Composition Including Glycerol Content

1. **Rationale:** We wanted to determine if the jerky treats contained chicken meat, as indicated on the label. Some products are actually composites of byproducts rather than slices of muscle. We found some products contained glycerin, although it was not listed on the label. We conducted additional testing for glycerin to follow up on this finding. Lysine testing was initiated to investigate if the condition in humans called “lysinuric protein intolerance (LPI)” could be involved in the jerky pet treat-associated illnesses. Many of the symptoms seen in humans with LPI, including Fanconi syndrome, are reported in dogs consuming jerky pet treats. Similar symptoms are also associated with oral ingestion of L-lysine in humans (Benning at al., 2007; Lo at al., 1996; Sebastio at al., 2011).

2. **Results:** More samples are pending testing. We tested most of the samples for glycerin, protein, moisture and fat content. We are continuing to test some of the samples for yeast, mold, crude fiber, fatty acid profile, lysine, and sulfite content.

In 2012, we evaluated 58 samples collected between 2009 and 2012, from 18 different brands. Seventeen of the 58 samples contained glycerin, but did not have glycerin on the label. A total of 9 different brands were mislabeled out of the 18 brands tested.

In 2013, we tested 40 samples taken from 14 different brands of jerky treats. Nine of the 40 samples contained glycerin. The nine positive samples came from 3 different brands of jerky treats, which were mislabeled.

C. Vitamin D Analysis

1. **Rationale:** Vitamin D over-supplementation can cause adverse effects on the kidney, including interfering with the ability of the collecting tubules to reabsorb water. Clinically, polyuria and hyposthenuria are observed. In dogs with Vitamin D-induced hypercalcemia, vasoconstriction of glomerular afferent arterioles occurs with a resultant decrease in glomerular filtration rate (Beasley, 1999). Due to previous reports of Vitamin D over-supplementation causing renal disease in pets, we believed investigation of this compound in jerky treats was warranted (USFDA d).

2. **Results:** We evaluated 19 samples in 2012. Vitamin D concentrations in 19 tested samples were in FDA-compliance. We have concluded this line of testing.

D. DNA Analysis

1. **Rationale:** FDA found that fish and other foods have been misbranded in the past. To make sure that jerky products are correctly labeled, we screened all store-bought and selected case samples (USFDA e). So far, we have found that chicken DNA is present in products labeled as “chicken” and duck DNA is present in products labeled “duck.” We are developing a protocol
and testing methods for DNA analysis of other potential contaminating products, including the toxic plant *Jatropha curcas*.

2. **Results:** Our DNA analysis of 70 store-bought samples and 22 consumer-complaint samples verified presence of DNA from the species on the label in all consumer-complaint samples and 68 of the store-bought samples. Results for two store bought samples did not comply with their label. The label claims for these two products stated that the treats were made from Tilapia, while our DNA analysis revealed the treats contained no Tilapia. DNA from other species was not excluded by these tests.

CVM generated a tissue and DNA database and DNA barcodes (DNA sequences) of the several *Jatropha* species. These data will be used in developing molecular *Jatropha* identification assays. CVM also developed methods for extracting DNA from *Jatropha* oil, for use in validating these assays.

III. **Chemical Toxicological Analysis**

**A. General Screens for Toxic Compounds**

1. **Rationale:** Consumers reported many different clinical signs. We selected specific chemicals for study based on their potential to cause renal disease, their association with Fanconi syndrome, or their potential to cause the gastrointestinal signs reported in many of the consumer complaints. We conducted a test that screens for a variety of chemicals of toxicological concern. After extraction, the samples are run on a gas chromatograph-mass spectrometer machine which compares the samples’ results to a library of information for known chemicals.

2. **Results:** We tested 31 samples and all were negative for the various toxic chemicals in the screen. The list of toxic chemicals we tested for is confidential.

**B. Metals and Elemental Analysis**

1. **Rationale:** Certain metals have been implicated in renal disease. Metals can be toxic at very low doses. Metals are nonbiodegradable, have long half-lives, and accumulate in tissues, leading to increased exposure in those who consume animals higher in the food chain. (Barbier et al., 2005; Johri et al., 2010). Metals can also cause kidney injury; Fanconi syndrome has been reported in copper storage disease (Appleman et al., 2008; Hill et al., 2008) and following cadmium exposure (Barbier et al., 2005). The degree of kidney injury is dependent on the nature, dose, route, and duration of exposure (Barbier et al., 2005). Diagnosing the source of the metals can be a problem; concentrations may be so low in food sources that samples test negative, while the patient’s tissues actually accumulate higher detectable levels (Thévenod F, 2003).

2. **Results:** We screened most of the store-bought samples to get an overview of the expected variability in non-case related jerky. We also compared multiple jerky strips from a single bag to evaluate the “within product” variability of metal content in the jerky. No metals have been found to be out of acceptable ranges in the store-bought jerky samples or the consumer-complaint cases. We also sampled for sulfur, which could be present as a contaminant if crude glycerin from biodiesel production was used to make the jerky treats.
As of May 7, 2013:

- We tested 8 consumer-complaint samples using Metals Screen 1 (Ag, As, BA, Be, Cd, Co, Cr, Cu, Ge, Hg, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sn, Ti, U, V, W, Zn). No metals or elements were present at toxic levels.
- We tested 71 store-bought samples and 18 consumer-complaint samples test using Metals Screen 2 (As, Ca, Cd, Ce, Co, Cu, Dy, Er, Eu, Fe, Gd, Ho, Hg, K, Mg, Mn, Mo, Na, Nd, P, Pb, Pr, Se, Sm, Ti, Zn). No metals or elements were present at toxic levels.
- We tested 41 consumer-complaint case samples and 4 store-bought samples using Metals Screen 3 (As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, Pb, S, Zn). No metals or elements were present at toxic levels.

We have recently sent 40 additional case-related samples for a metals screen which includes sulfur. As already mentioned, sulfur can enter the food production chain in several ways and since it can cause hypersensitivity responses in the form of sulfites (sulfite, bisulfite, metabisulfite and sulfur dioxide), we are using the metals-elemental analysis as a first test to screen samples for these compounds.

C. Glycols

1. Rationale: Several organic compounds have been associated with renal failure and Fanconi’s syndrome, including ethylene glycol (EG) (O’Brien et al., 1998; ATSDR, 1997; Alfred et al., 2005; Rollins et al., 2002; Brent et al., 1999; Eder et al., 1998), and diethylene glycol (DEG). In 2006, glycerin contaminated with DEG, was used to produce pharmaceuticals and caused renal failure in humans (Wax, 1995; Rentz et al., 2008). Because some jerky treat products list glycerin as an ingredient, we wanted to rule out contamination with either DEG or EG. Recently, propylene glycol was reported to cause intoxication in a dog, so we have now included it in our screen (Claus et al., 2011). Christopher et al. (1989, 1990) found that ingestion of propylene glycol causes lactic acidosis. Two Vet-LIRN laboratories tested jerky pet treat products for ethylene glycol, diethylene glycol, and propylene glycol. To compare methods, we broke 11 samples into pieces. We then divided the pieces from the same treat into three subs for testing (a third sub was archived.) Remaining samples were tested only by Lab 1. The same labs were asked to develop methods for dihydroxyacetone and 1, 3 propanediol. As with previous testing, we broke 8 different samples into pieces. We divided pieces from the same treat into three subs (we archived the third sub).

2. Results: From 2011 to 2012, Lab 1 tested 31 samples for diethylene glycol and ethylene glycol. All samples tested negative. Lab 1 also tested 21 samples for propylene glycol. One sample tested positive (170,000 ppm). The lab also tested 9 samples for dihydroxyacetone and 1, 3 propanediol. All samples tested negative.

From 2011 to 2012, Lab 2 tested 11 samples for diethylene glycol and ethylene glycol. All samples tested negative. Lab 2 also tested 19 samples for propylene glycol. Two samples tested positive, 25 ppm and 25,262 ppm, respectively. The lab also tested 8 samples for dihydroxyacetone and 1, 3 propanediol. One sample tested positive for 1, 3 propanediol.

D. Glycerin Metabolites
1. **Rationale:** The clinical signs noted in the consumer complaints have been very similar to those associated with antifreeze (ethylene glycol) poisoning. Ingested ethylene glycol is converted to glycolaldehyde to glycolic acid to glyoxylic acid to oxalic acid to calcium oxalate. We are exploring the possibility that during manufacturing process of jerky pet treats, irradiation, in combination with other factors, produces toxic metabolites of ethylene glycol from glycerin that also converted to calcium oxalate. In 2013, we began developing a method to detect these glycerin metabolites.

2. **Results:** We have tested jerky treats for ethylene glycol and all samples were negative. Testing is pending for toxic metabolites of ethylene glycol from glycerin.

**E. Sugar Alcohols**

1. **Rationale:** Glycerin can be converted to xylitol (Znang at al., 2011). Xylitol administration was associated with the production of calcium oxalate crystals in the kidney of a human patient (Evans, 1973), and with liver failure in dogs (Dunayer, 2006). Sorbitol is a sugar substitute, testing is performed in order to establish if it’s being added to the treats without proper labeling.

2. **Results:** Testing is pending.

**F. Other Organics**

1. **Rationale:** Hexachlorobutadiene has been used as fungicide and is also an environmental contaminant (Fattore at al., 1998). Accidental or experimental exposure has been associated with kidney failure and elevated urine glucose (Chiusolo et al., 2010; Kirby and Bach, 1995; Staples et al., 2003; Berndt and Mehendale, 1979; Hook et al., 1982). Since cooking oils in China have been reportedly contaminated with a variety of organic contaminants (Taipei Times, 2010) this compound was included in our testing program. Paraquat ingestion has also been reported to induce Fanconi’s syndrome in humans (Gil et al., 2005). Aristolochic acids are hypothesized to be causative agents in Balkan endemic nephropathy and a related, possibly identical, condition known as "Chinese herbs nephropathy" (USDA f; Gluhovschi et al. 2011).

2. **Results:** Lab 2 tested 11 samples (2 control and 9 case-related samples) for hexachlorobutadiene. All samples were negative. Lab 1 tested 17 samples (3 control, 12 case-related, and 2 store-bought) for paraquat. All samples tested negative. Two different labs tested 27 samples for aristolochic acid. All samples tested negative.

**G. Antibiotics**

1. **Rationale:** A number of drugs have been associated with kidney injury and glucose in the urine. Drugs such as gentamicin and tetracycline degradation products (epitetracycline, anhydrotetracycline, and epi-anhydrotetracycline), can cause proximal renal tubule reabsorption abnormalities (Brown et al., 1986; Brown et al., 1989). Gentamicin or other aminoglycoside therapy has been associated with acute kidney failure and Fanconi syndrome in dogs and humans (Brown et al., 1985, Ghiculescu and Kubler, 2006). These antibiotics are, however, given parenterally and are unlikely to result in kidney damage if eaten in a jerky treat. Degradation products of tetracycline can also cause a transient nephropathy in humans (Frimptere et al., 1963; Gros, 1963) and a Fanconi-like syndrome in rats, (Lindquist et al., 1966); thus, we have been testing treats for these compounds.
2. Results:
   a. New York State Laboratory
   The New York State Laboratory tested 69 Pieces from 9 cases and 50 pieces from 5 store-bought bags (total 119 pieces from 14 samples) for various sulfa drugs and tetracycline degradation products. The lab tested multiple pieces from each case (5-10) to evaluate the variability among different pieces of chicken breast within each bag. Results showed very low (parts per billion) residues in some of the samples; concentrations were well below established tolerance levels. The highest tetracycline product result was 0.04 ppm (tolerance 2 ppm) with 7 positive samples (20 of the 119 pieces were positive, and all positive pieces came from 7 different samples).

   The lab also detected several sulfa drugs. Sulfaquinoxaline (tolerance 0.1 ppm) was found in 9 samples (27/119 pieces). The highest concentration detected was 0.041 ppm. One sulfa drug for which no tolerance level exists, sulfaclozine, was found in 7 samples (30/119 pieces). The highest concentration detected was 0.257 ppm.

   Enrofloxacin was found in 4 samples (9/119 pieces). The highest concentration was 0.132 ppm. Tilmicosin was found in 2 samples (9/50 pieces). The highest concentration was 0.005 ppm. The levels of antibiotics found in these products are unlikely to cause the clinical signs reported in the affected animals.

   b. CVM Vet-LIRN Laboratory Testing
   Lab 1 tested 27 samples for gentamicin. All samples were negative. The lab also tested the treats for the presence of the sulfa-drugs sulfadiazine, sulfamerazine, sulfadimethoxine, sulfamethazine, sulfapyridine, sulfaquinoxaline, and sulfathiazole. All samples were negative.

   Lab 2 tested 11 samples for sulfa-drugs. Four samples tested positive for sulfanilamide (range 0.06-0.232 ppm). The lab also tested for sulfamethoxazole, sulfamethazine, sulfadiazine, sulfaquinoxaline, sulfathiazole, and sulfadimethoxine and these were all negative.

H. Other Drugs--Monensin
   1. Rationale: Monensin sodium is an ionophore coccidiostat agent that is used as a feed additive in chickens. Hazlett et al. (1992) described its toxicity in dogs following ingestion of dog food contaminated with a poultry premix containing monensin, roxarsone, and zinc bacitracin. Clinical signs included polydipsia, polyuria, dark urine, and vomiting. It was suspected that the combined action of monensin and arsenic produced the renal medullary necrosis found at necropsy. We therefore evaluated the potential for the use (or overuse) of monensin in chickens to cause residues in jerky treats.

   2. Results: A Vet-LIRN lab tested 27 samples for monensin (5 controls, 20 case-related, and 2 store-bought samples). All samples tested negative.

I. Biogenic Amines
   1. Rationale: Biogenic amines were included in our screen because some of these compounds, like spermine, have caused impaired kidney function, kidney histopathological changes, and changes in blood plasma electrolytes and urea
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(Radosevich, 2007; Til et al., 1977). These substances could be present in live poultry before slaughter, or could end up in a final jerky treat product due to chicken meat contamination or degradation prior to, or during, the manufacturing process. These amines could also be markers of improperly stored or prepared chicken meat. We tested selected treats for biogenic amines to determine whether the jerky could have come from degraded chicken bodies.

2. Results: A Vet-LIRN lab tested 27 samples for various biogenic amines (putrescine, cadaverine, histamine, agmatine, spermidine, and spermine). Putrescine was detected in 2 case-related samples and 2 controls (30-80 ppm). Cadaverine was found in 3 control samples, 12 case-related samples, and 2 store-bought samples (10-2190 ppm). Histamine was detected in small concentrations (1-35 ppm) in each group of samples (11 samples). Agmatine was found in 5 samples, 3 control samples and 2 case related samples (1-500 ppm). Spermidine was detected in small concentrations (12-50 ppm) in almost all of the tested samples. Spermine was found in all samples, in concentrations ranging from 30-320 ppm. Biogenic amines were not included in 2013 testing plans because we were unable to find a connection between jerky pet treat-related illness and the reported levels of biogenic amines found in tested jerky pet treats. We decided to give priority to other compounds that could cause the signs associated with the reported illnesses.

J. Phorbol Esters

1. Rationale: We investigated toxins from the plant, *Jatropha curcas*, due to the potential for glycerin to be contaminated with these toxins if the source of glycerin was from biodiesel production using Jatropha oil. In 2012, FDA issued a notice to industry (FDA d) regarding oils, glycerin, and proteins commonly used in the production of FDA-regulated products, due to their potential for contamination with these toxins if the ingredients are derived from the *Jatropha* plant.

2. Results: There are currently no standards for these toxins and we are in the early stages of method development for their detection. We evaluated samples for the presence of *Jatropha* factors. Ten consumer-complaint jerky treat samples showed unknown peaks of different intensity with Q-Exactive High resolution mass spectrometer and AB SCIEX 4000 triple quadruple mass spectrometer, with the 311 cluster absent. *Jatropha* factors were not detected at levels above 0.050 ppm.

In addition, we used an optimized method to extract *Jatropha* factors from seeds and oil of *Jatropha curcas* with methanol. Jatropha factors were then characterized and quantitated. Our goal was to produce an enriched extract of these factors from oil and plant seeds to serve as a crude standard with which to screen glycerin and food products for these toxins. We will test glycerin further for contaminants as methods are developed and refined.

K. Additives/Preservatives

1. Nitrites
   a. Rationale: Nitrites are sometimes used for preservation and may be present in excess quantities. If industrial salts are used, nitrites may be elevated. At very high concentration, nitrites can cause toxicity.
b. Results: All 42 tested samples were negative for the presence of nitrite salts. We have no plans for further nitrite testing.

2. Sulfites
   a. Rationale: Sulfonamide sensitivity could potentially be a sulfite sensitivity. The higher sulfur levels on the jerky treats may be caused by dipping the chicken breasts and/or sweet potatoes in a sulfite-containing solution to preserve the products’ color.
   b. Results: We found sulfonamide residues in multiple jerky pet treats (see antibiotic section). Testing is pending for 40 samples.

L. Tanning Agents
   1. Rationale: In 2011, Fox News reported that leather protein was being used to adulterate milk in China. Such reports raise the possibility of other products being used in jerky as a substitute for chicken muscle. As a result, selected new cases will also be screened for evidence of tanning agents such as tannin or gallic acid.
   2. Results: Lab 1 tested 17 samples (3 controls, 12 case-related, and 2 store-bought) for gallic and tannic acid. All samples were negative.

M. Flavoring Agents
Since some jerky treats are labeled with unspecified “spices or flavorings,” we may evaluate selected chemicals which have been associated with kidney injury or Fanconi syndrome. These chemicals include monosodium glutamate (MSG), (Vinodini et al., 2010), maleic acid (Gmaj et al., 1973; Al-Bander et al., 1982, 1985; Worthen, 1963; Hoppe et al., 1976; Bank et al., 1986, Brewer et al., 1993), and methyl-4-pentenoate (Gougoux et al., 1989). We will need to develop or bridge some of the methods for these agents to the jerky matrix.

1. Malic Acid, Maleic Acid
   a. Rationale: Maleic acid has been shown to cause a Fanconi-like syndrome in dogs after being given intravenously (Al-Bander et al., 1982, 1985). Malic acid is also a food additive. We are testing the treats to see if it is being added to them without proper labeling.
   b. Results: A Vet-LIRN lab tested 27 samples in 2012. All samples tested negative for maleic acid. Three consumer complaint-related samples tested positive for malic acid at levels considered non-toxic. 2013 Results are pending for malic and oxalic acid.

2. Fumaric acid
   a. Rationale: Fumaric acid is also a food additive. Like malic acid, we test products to see if it’s being added to the treats without proper labeling. Fumaric acid is cleared in the U.S. by the USDA’s Meat and Poultry Inspection Division as a curing accelerator used only in combination with curing agents to accelerate color fixing in cured, comminuted meat; meat food; poultry, or poultry products, at a level of 0.065 percent (or 1 oz per 100 lbs) by weight of the meat, meat byproducts, poultry, or poultry by-products before processing (The Food Chemical News Guide). Fumaric acid, upon heating, converts to the irritant maleic anhydride, which then hydrolyzes to maleic acid (Huntsman Petrochemical Corporation).
b. **Results:** We tested 20 consumer-complaint related samples and only one tested positive (reporting limit: 60 ppm).

3. **Methyl-4-pentenoate**
   a. **Rationale:** Methyl-4-pentenoate (4-pentenoic acid) is listed under 21CFR 172.515 as an approved synthetic flavoring substance in the U.S. We may test jerky treats for methyl-4-pentenoate (and possibly similar compounds listed under 21CFR 172.515) to determine if treats were coated in these compounds to enhance flavor.
   
   b. **Results:** We must first develop a suitable method to detect this.

N. **Illegal Dye Agents**

1. **Rationale:** Reports of adding Sudan dyes to duck feed to color the yolks red surfaced in 2006 (Centre for Food Safety, Hong Kong). In rare cases of ingestion, kidney failure has also been reported (Sahay et al., 2009; Suliman et al., 1983). Because animals can have idiosyncratic or hypersensitivity responses to dyes and because other general food safety concerns exist for dyes, such as carcinogenicity (Stiborová et al., 2002), we are screening some of our samples for these substances.

2. **Results:** Seven consumer complaint-related samples were tested for 19 dyes including: Auramine, Bixin, Butter Yellow, Fast Garnet, Metanil Yellow, Orange II, Orange Oil SS, Para Red, Rhodamine B, Sudan Black B, Sudan I-IV G, Sudan Orange, Sudan Red 7B, Sudan Red B, Sudan Red G and Toluidine Red. All samples tested negative. We have no plans for further dye testing.

IV. **Evaluation of Jerky Treat Irradiation**

Manufacturers use irradiation, an approved process, on a broad variety of food products to ensure food safety and provide insect control. FDA can verify absorbed radiation doses through a variety of methods, such as measuring furan concentration and monitoring levels of 2-acylcyclobutanones (2-ACB).

A. **Furan Analysis**

1. **Rationale:** Many of the treats are irradiated; items that are improperly-irradiated may contain marker chemicals indicating improper handling. In general, the literature indicates that irradiation of food does not negatively affect dogs. Blood et al. (1966) reported; however, that dogs fed a high-dose irradiated pineapple jam diet developed glucose in the urine. We therefore evaluated jerky samples for evidence of high-dose irradiation.

2. **Results:** We analyzed 9 samples (7 chicken, 1 duck, and 1 yam treat). Furan concentrations were in compliance. We have concluded this line of testing.

B. **2- Alkylcyclobutanone (2-ACB) Analysis**

1. **Rationale:** While the correlation of 2-ACB levels in raw muscle foods has been well documented, the literature provides little information on the effect of processing and various additives on observed 2-ACB levels. We believe that jerky pet treat products are irradiated at doses of about 10 kGy, though we do not have any current verification tests. Also, we do not know what, if any, effect the glycerin treatment prior to irradiation has on the final product.
2. **Results:** We conducted a preliminary study to measure the levels of 2-dodecylcyclobutanone (2-DCB) in chicken jerky treats soaked in glycerin for varying amounts of time (0 control, 20 min., and 24 hours) and subsequently irradiated at different doses (0 control, 5, 10, 15, and 25 kGy). Chicken jerky samples were prepared from store-bought chicken breast tenderloins, stored in Whirl-Pak bags, and then irradiated with a Cesium-137 source, at 200°C. 2-DCB is a common 2-ACB found in muscles eaten for food which contain palmitic acid. Our study showed that 2-DCB could be used to monitor the absorbed radiation dose and that soaking the chicken in glycerin at different times did not affect the detection of 2-DCB. A second phase of our study is pending and includes consumer complaint-related samples.

V. **Rationale for Continued Product Testing**

FDA is currently collecting jerky samples from recent consumer complaint cases in which the pets had severe illnesses, such as kidney failure or other organ failure. We plan to test the new samples for the above-listed array of chemicals. In order to have confidence in negative results, we need to test a large number of samples. We recognize that in the past, sample size has been an issue. If a toxic chemical (or multiple toxic chemicals) is present intermittently, and perhaps only present in some jerky pieces within a product bag, the chances of having false negative test results are relatively high. We are trying to increase our sample numbers, although the number samples can still be a problem if the owner only has a few pieces left. Most of the 2013 case samples are currently being submitted to laboratories.

*Note: Contracts for testing were not awarded until April 2013, due to a continuing resolution and budgetary uncertainties.* Starting in April 2013, batches containing 20 samples are being sent every 2-3 months to the contract laboratories.

VI. **Diagnostic Sample Testing**

Diagnostic testing, such as blood chemistry, urinalysis, and complete blood count can provide important information about the affected animals’ illnesses. In cases where the animals die, a necropsy (an animal autopsy) can fill in the gaps in our information, helping us pinpoint a cause for the reported jerky treat injuries and deaths. The results may help us target specific toxic chemicals we should look for in the jerky treat products.

In most jerky treat cases, affected animals do not die (Hooper et al., 2011). In the past, necropsies were rarely done in cases where the animals died. CVM is reaching out to owners of affected dogs undergoing treatment to obtain diagnostic samples from these animals. CVM will pay for additional testing (preauthorized). If an animal dies, CVM will pay for the animal’s necropsy, histopathology, and tissue residue testing. We have listed the results from our diagnostic sample testing below.

We are requesting preserved kidney specimens from reported cases when available. We are conducting additional tests on these samples, including making new slides for histologic confirmation of pathology findings and creating special tissue sections on quartz for Raman Spectrographic analysis. These cases are currently still under investigation.

We are consulting with a number of board-certified veterinary toxicologists around the country for their recommendations on additional potential toxic chemicals we should test for. Some of these tests may require significant method development and method expansion from one food type to the jerky food type for the testing to be valid. We may need to set up contracts with several laboratories to accomplish this.
A. Gross Necropsy
As of June 5, 2013, gross necropsies were done in 27 cases. Of these, Vet-LIRN labs performed 4 necropsies, Vet-LIRN coordinated 6 necropsies with referring veterinarians and owners, and the remaining necropsies were completed by the time CVM received the complaints.

B. Histopathology
As of June 5, 2013, Vet-LIRN labs performed histopathology in 8 cases. Non-Vet-LIRN labs conducted additional histopathology in 17 cases.

C. Blood Chemistry
As of June 19, 2013, Vet-LIRN ordered routine blood testing on 9 cases.

D. Urinalysis
As of June 19, 2013, Vet-LIRN ordered urinalyses on 3 cases.

E. Raman Spectroscopy
Of the 25 cases where histopathology was reported to Vet-LIRN, 5 had noted crystal formation in the kidneys. Vet-LIRN performed Raman analysis on these 5 cases. In one case, no crystals were identified. In the 4 other cases, Vet-LIRN identified calcium oxalate crystals. We do not know the cause of these crystals.

F. Fanconi Urine Panel
As of June 5, 2013, Vet-LIRN tested 45 case-related samples using a Fanconi urine panel. Twenty-nine samples tested positive, 4 were reported as probably positive, and 12 samples tested negative. We are beginning follow-up testing on selected cases. We will collect new urine samples 7-8 weeks after the original collection and we will re-test them using the Fanconi urine panel. We are trying to determine how long Fanconi panel markers remain in the affected dog’s urine after treats are no longer fed.

VII. Collaborating Laboratories
Many laboratories around the country are collaborating with this investigation. These laboratories include the California Animal Health and Food Safety Laboratory System at University of California Davis, the Animal Health Diagnostic Center at Cornell University, the Pennsylvania Animal Diagnostic Laboratory System New Bolton Center, the Metabolic Genetics Laboratory at the University of Pennsylvania, the Ohio Department of Agriculture Animal Disease Diagnostic Laboratory, the Animal Disease Research and Diagnostic Laboratory at South Dakota State University, the Analytical Sciences Laboratory at the University of Idaho, the Breathitt Veterinary Center at Murray State University, Kansas State University, and USDA-Agriculture Research Service in Pennsylvania. Sixteen Vet-LIRN laboratories also participated in the initial collection and screening of jerky treats for Salmonella.

About the Veterinary Laboratory Response Network (Vet-LIRN)
During the melamine episode in 2007, CVM identified a major gap in the ability of the FDA to obtain or share information with state and academic veterinary diagnostic laboratories responding to chemical and microbial feed or drug contamination events. In 2010, CVM obtained funding to create a Veterinary Laboratory Investigation and Response Network (Vet-LIRN), to partner with veterinary diagnostic laboratories in documenting, investigating, and diagnosing animal feed, pet food, and animal drug-related illnesses.
During its first year, Vet-LIRN recruited a Program Director and collaborated with the existing Food Emergency Response Network (FERN; http://www.fernlab.org/). In March 2011, CVM and laboratory directors held a development meeting to exchange ideas and information. During Summer 2011, Vet-LIRN developed its infrastructure, and by August 16, 2011, laboratories had joined the network and began collaborating on investigations. Two priority projects were also developed in collaboration with FERN, one focusing on chemical adulterants and another on microbial contamination of animal feed.

**Microbiological contamination.** In collaboration with FERN, Vet-LIRN is working with six food-testing laboratories, conducting tests on four different animal feed pathogens that compromise feed safety. The testing data will help CVM prioritize the Center’s surveillance efforts and will increase the capabilities of field laboratories to work with some of the more unusual matrices encountered in animal feed, such as dried or semi-soft items.

**Chemical contamination.** In collaboration with USDA’s Food Safety Inspection Service (FSIS), Vet-LIRN is working with three chemistry laboratories to develop ways to detect melamine and cyanuric acid in pig tissues such as ham, pork loin, and kidney. The new methods will increase the Agencies’ preparedness in case of melamine feed contamination.

**Proficiency testing.** The FDA’s Center for Food Science and Applied Nutrition and CVM are working with the Institute for Food Safety and Health and the Illinois Institute of Technology Moffett Campus, to provide chemical and bacterial proficiency testing for Vet-LIRN labs. The testing will document the labs’ readiness for, and accuracy in, conducting various chemical and bacterial diagnostic tests.

**Salmonella presence.** Vet-LIRN signed its first 11 cooperative agreements with state and university diagnostic laboratories to evaluate *Salmonella* prevalence in dog and cat diagnostic samples. This initiative results from the increasing number of human *Salmonella* outbreaks attributed to pet treats and pet foods. The studies will characterize Salmonellosis in dogs and cats. CVM will use the data to prioritize our investigations of food-borne diseases that adversely affect animal and human health.

**Identification of contaminants.** Vet-LIRN awarded a contract to the University of California’s Davis Diagnostic Laboratory to determine which food safety contaminants (bacterial and chemical) veterinary diagnostic laboratories have encountered since 2006.

**Diagnostic samples.** Vet-LIRN awarded a contract to the Iowa State Veterinary Diagnostic Laboratory to provide diagnostic samples and coordinate activities among the American Association of Veterinary Diagnostic Laboratories, the Vet-LIRN program office, the Institute for Food Safety and Health, the Illinois Institute of Technology Moffett Campus, and other Vet-LIRN diagnostic laboratories.

Since its inception, Vet-LIRN has investigated numerous cases of suspected feed-associated illnesses and has collaborated with CVM’s Complaint Emergency Recall Team to evaluate which cases should receive further testing. Vet-LIRN reviewed over 40 adverse event cases during the 2011 fiscal year and analyzed a number of specimen samples from these cases. Vet-LIRN followed up on four official case investigations in Fiscal Year 2011, and 22 in Fiscal Year 2012. This fiscal year, Vet-LIRN is working on 17 official case investigations. Beside official case investigations, Vet-LIRN also evaluates many adverse events and gives comments to the Emergency Recall Team.

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2 *The Food Emergency Response Network (FERN)* integrates the nation’s food-testing laboratories at the local, state, and federal levels into a network that is able to respond to emergencies involving biological, chemical, or radiological contamination of food. FERN is jointly sponsored by the FDA and U.S. Department of Agriculture.
As a result of recruiting and networking efforts, Vet-LIRN had 22 full-member veterinary diagnostic laboratories at the end of 2011. As of May 2013, we have 34 member veterinary diagnostic laboratories, as listed and shown in the map below.

1. Arkansas: Arkansas Livestock and Poultry Lab
2. California: California Animal Health and Food Safety Laboratory at University of California, Davis*+
3. Canada: University of Guelph, Animal Health Laboratory
4. Colorado: Colorado State University, Colorado State University Diagnostic Laboratory*+
5. Connecticut: University of Connecticut, Veterinary Medical Diagnostic Laboratory+
6. Georgia: Athens Veterinary Diagnostic Laboratory at University of Georgia*+
7. Georgia: University of Georgia, Tifton Veterinary Diagnostic and Investigational Laboratory+
8. Indiana: Animal Disease Diagnostic Laboratory, Purdue University+
9. Iowa: Department of Veterinary Diagnostic and Production Animal Medicine at Iowa State University*+
10. Kansas: Kansas State University Veterinary Diagnostic Laboratory
11. Kentucky: Breathitt Veterinary Center; Murray State University+
12. Kentucky: University of Kentucky; Department of Veterinary Sciences; Veterinary Diagnostic Laboratory+
13. Louisiana: Louisiana State University, School of Veterinary Medicine, Department of Pathobiological Science+
14. Maryland: Maryland Department of Agriculture, Frederick Animal Health Laboratory
15. Michigan: Diagnostic Center for Population and Animal Health at Michigan State University+
16. Minnesota: Veterinary Diagnostic Laboratory at University of Minnesota
17. Mississippi: Mississippi State University, Veterinary Research and Diagnostic Lab System+
18. North Carolina: North Carolina State College of Veterinary Medicine
19. North Carolina: United States Department of Agriculture, Agricultural Marketing Service Laboratory Division
20. New Jersey: New Jersey Department of Agriculture; Division of Animal Health+
21. New York: Animal Health Diagnostic Center; College of Veterinary Medicine; Cornell University+
22. North Dakota: North Dakota State University; Veterinary Diagnostic Laboratory
23. Ohio: Ohio Animal Disease Diagnostic Laboratory at the Ohio Department of Agriculture*+
24. Oklahoma: Oklahoma State University, Oklahoma Animal Disease Diagnostic Laboratory
25. Oregon: Oregon State University; Veterinary Diagnostic Laboratory+
26. Pennsylvania: University of Pennsylvania, Pennsylvania Animal Diagnostic Laboratory, New Bolton Center*+
27. South Dakota: Animal Disease Research and Diagnostic Laboratory at South Dakota State University*+
28. Tennessee: Tennessee Department of Agriculture, Kord Animal Health Diagnostic Laboratory
29. Texas: Texas A&M University, Clinical Microbiology Laboratory
30. Utah: Utah Veterinary Diagnostic Laboratory; Utah State University
31. Virginia-Maryland: Virginia Maryland Regional College of Veterinary Medicine, Virginia Tech Animal Laboratory Services
32. Washington: Washington State University, College of Veterinary Medicine, Washington Animal Disease Diagnostic Laboratory
33. Wisconsin: University of Wisconsin Madison, Wisconsin Veterinary Diagnostic Laboratory
34. Wyoming: Department of Veterinary Sciences at University of Wyoming

*Laboratories that received funding through RFA-FD-11-010: Evaluation of Salmonella in Symptomatic and Asymptomatic Pets: Study for the Vet-LIRN Program
+Laboratories that received funding through PA-12-194: CVM Vet-LIRN Veterinary Diagnostic Laboratory Program
References


Til HP, Falke HE, Prinsen MK, and Willems MI. Acute and Subacute Toxicity of Tyramine,Spermidine, Spermine, Putrescine and Cadaverine in Rats. *Food and Chemical Toxicology* 1977; 35:337-348.


USFDA e. DNA-based Seafood Identification (http://www.fda.gov/Food/FoodScienceResearch/DNASeafoodIdentification/default.htm), 2011. (Accessed 07-22-2013)


