

# Evaluation of the association between feeding raw meat and *Salmonella enterica* infections at a Greyhound breeding facility

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**Objective**—To investigate *Salmonella enterica* infections at a Greyhound breeding facility.

**Design**—Cross-sectional study.

**Animal and Sample Populations**—138 adult and juvenile dogs and *S enterica* isolates recovered from the dogs and their environment.

**Procedures**—The investigation was conducted at the request of a Greyhound breeder. Observations regarding the environment and population of dogs were recorded. Fecal, food, and environmental specimens were collected and submitted for *Salmonella* culture. Isolates were serotyped and tested for susceptibility to 16 antimicrobials. Isolates underwent genetic analyses by use of pulsed-field gel electrophoresis and ribotyping.

**Results**—*S enterica* was recovered from 88 of 133 (66%) samples of all types and from 57 of 61 (93%) fecal samples. Eighty-three (94.3%) of the isolates were serotype Newport, 77 (87.5%) of which had identical resistance phenotypes. Genetic evaluations suggested that several strains of *S enterica* existed at the facility, but there was a high degree of relatedness among many of the Newport isolates. Multiple strains of *Salmonella enterica* serotype Newport were recovered from raw meat fed on 1 day.

**Conclusions and Clinical Relevance**—*S enterica* infections and environmental contamination were common at this facility. A portion of the *Salmonella* strains detected on the premises was likely introduced via raw meat that was the primary dietary constituent. Some strains appeared to be widely disseminated in the population. Feeding meat that had not been cooked properly, particularly meat classified as unfit for human consumption, likely contributed to the infections in these dogs. (*J Am Vet Med Assoc* 2006;228:1524–1532)

Many high-performance dogs such as racing Greyhounds and sled dogs are fed high-protein

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Supported by the James L. Voss Veterinary Teaching Hospital and by the Animal Population Health Institute through a grant from the United States Department of Agriculture Cooperative State Research, Education, and Extension Service (USDA:CSREES). The authors thank Denise Bolte and Leena Jain for technical support. Address correspondence to Dr. Morley.

## ABBREVIATIONS

PFGE Pulsed-field gel electrophoresis  
ATCC American Type Culture Collection

diets that consist mostly of raw meat.<sup>1-5</sup> Because of cost considerations, meat products used for this purpose are often of low quality and have been classified and labeled as unfit for human consumption. Some of these products consist of muscle, organ meats, offal, and other tissues from mammals or poultry that died prior to slaughter or were condemned when inspected at slaughter.<sup>2</sup> Although such meat is an inexpensive source of protein, it has the potential to expose animals to many pathogenic microorganisms, including *Salmonella* spp, *Campylobacter jejuni*, and *Escherichia coli*, especially when fed raw. *Salmonella* spp is often detected in raw meat fed to dogs and in their feces,<sup>5-9</sup> and outbreaks of salmonellosis are reportedly<sup>3</sup> common among Greyhounds in racing kennels.

In 2001, veterinarians from Colorado State University investigated an outbreak of diarrheal disease and death among young puppies at a Greyhound breeding facility. Owners requested that investigators assess potential sources of *Salmonella enterica* infections and estimate the degree of dissemination of contamination at the facility and infections among the population.

## Materials and Methods

**Overview**—Prior to requesting the investigation by Colorado State University veterinarians, the referring veterinarian had submitted 3 puppies that died for necropsy at the university Veterinary Diagnostic Laboratory. Evidence of septicemia, enteritis, and colitis was observed at necropsy, and *S enterica* was cultured from the small intestine of 2 of the puppies. Detailed historical information was obtained from the referring veterinarian and the owners of the facility prior to conducting a site visit; during the site visit, routine operations were observed and samples of feed and feces were collected, and those samples and environmental swab specimens were submitted for culture for *S enterica*. Isolates obtained from cultures were analyzed for antimicrobial susceptibility and were genetically characterized to determine whether there was evidence of multiple strains.

**Field investigation**—The apparent outbreak was discussed with the referring veterinarian and owners by telephone prior to visitation of the premises. During these conversations, the owners were asked to collate information related to management of dogs on the premises, occurrence of illness during this outbreak, and treatment and disposition of affected animals. Investigators visited the breeding operation to collect available records, discuss the outbreak with owners and the referring veterinarian, observe management

procedures, and collect specimens for culture. A report summarizing investigators' observations and recommendations as well as results of microbiologic investigations was issued to the owners and referring veterinarian. Maps of the facility were prepared during the site visit and were used as a key for identifying sampling locations (Figure 1).

**Samples**—The sampling strategy was formed on the basis of 2 goals. First, swab specimens of feces, soil, the environment, cleaning tools, and water buckets were collected to evaluate the extent of environmental contamination and dissemination of *S enterica* in the population. Second, raw meat and mixed feed were collected to determine whether *S enterica* infections were originating from those sources. Fecal samples ( $n = 61$ ) were collected from the floors of pens housing all age groups (approx 5 samples/enclosure). Soil samples ( $n = 19$ ) were collected from outside housing areas by scraping surfaces with collection tubes. Samples of the environment ( $n = 31$ ) were collected from the floors of indoor housing areas, surfaces of sinks, surfaces of water buckets, and surfaces of cleaning tools by use of sterile sponges premoistened with neutral buffered saline (0.9% NaCl) solution.<sup>a</sup> One pooled sample of approximately 6 flies was collected from indoor areas. Feed samples included frozen raw beef (8 samples from brand A and 8 from brand B), drippings or meat juice from thawing beef (2 samples from brand B) collected prior to mixing of daily rations, and mixed diet collected from food bowls that had already been presented to the dogs (3 samples) from various locations in the facility. Production lot information was not available on beef packages, but samples were all obtained from single shipments of brand A and brand B products. Although raw tripe was also commonly included as a dietary constituent, none was available at the facility on the day of the site visit. New gloves were donned prior to collecting all samples. Samples were transported on ice to the Colorado State University Veterinary Diagnostic Laboratory for culture of *Salmonella* spp within 6 hours of collection.

**Culture of *S enterica***—Feces, meat, and meat drippings (1 g or 1 mL) were placed in 10 mL of tetrathionate broth with iodine<sup>b</sup> and incubated at 42°C for 18 hours. Flies and sponges used for environmental and soil samples were placed in 10 mL of thioglycollate broth and incubated at 42°C for 48 hours. After incubation, enriched samples were plated on XLT4 agar<sup>c</sup> and incubated at 37°C for 24 to 48 hours. Plates were evaluated after 24 and 48 hours of incubation and hydrogen sulfide-producing colonies were further evaluated. Suspect isolates were plated on trypticase soy agar with 5% sheep red blood cells<sup>d</sup> and incubated at 37°C for 16 hours. Isolates were evaluated with *Salmonella* poly-O antisera and group-specific antisera. Isolates that reacted with both poly-O and group-specific antisera were assigned a presumptive identification of *S enterica*. Those isolates were sent to the USDA–National Veterinary Services Laboratories, Ames, Iowa, for serotyping and to the USDA–Agriculture Research Service's Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Athens, Ga, for antimicrobial susceptibility testing and genetic analyses.

**PFGE**—*Salmonella enterica* isolates were evaluated by use of PFGE. The 24-hour *S enterica* PFGE procedure was performed as described by the National Molecular Subtyping Network for Foodborne Disease Surveillance (Pulse Net).<sup>10</sup> Briefly, whole-cell DNA plugs embedded in 1.0% agarose<sup>e</sup> were digested with 10 units of the selected restriction enzyme (*Xba*I).<sup>f</sup> The DNA standard was prepared from *S enterica* serotype Newport AM01144, and digested DNA was separated by use of a PFGE system as per manufacturer's instructions.<sup>8</sup> Electrophoresis was carried out for 19 hours at 6 V by use of 2.2 L of a buffer<sup>h</sup> at a temperature of 14°C and

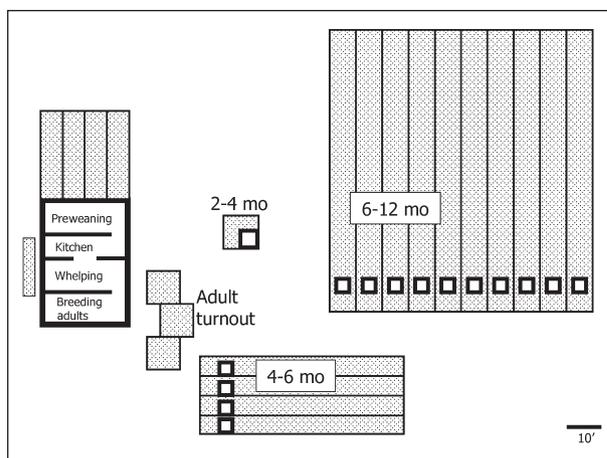


Figure 1—Spatial diagram of Greyhound breeding facility (scale is approx). Thick borders represent buildings and shelters. Stippled areas represent outdoor enclosures. Labels refer to ages of dogs housed in a given area.

an initial pulse time of 2.16 seconds followed by a final switch time of 63.8 seconds. Genetic analysis software<sup>1</sup> was used to normalize the band patterns for comparison with PFGE profiles. Reference strains of *S enterica* serotype Typhimurium DT104 and *E coli* were included in the genetic analyses for comparison.

**Ribotyping**—*Salmonella enterica* isolates were evaluated via ribotyping by use of an automated ribosomal microbial characterization system<sup>1</sup> as per manufacturer's instructions. Briefly, colonies were removed from 24-hour sheep blood agar plates, cells were lysed, and DNA was digested with the restriction enzyme *Pvu*II. Samples were loaded into the ribosomal microbial characterization system, and restriction fragments were separated via electrophoresis and transferred to a nylon membrane. A DNA probe for *Salmonella* spp was hybridized to the genomic DNA of each isolate on the membrane. Bound, labeled antibodies were captured with a chemiluminescent detection system containing a charged-coupled device camera. Once analyzed, band patterns were assigned to ribogroups. On the basis of normalized band patterns, genetic analysis software<sup>1</sup> was used to construct a dendrogram using Pearson's correlation coefficient to determine genetic relatedness. Reference strains of *S enterica* serotype Typhimurium DT104 and *E coli* were included in the genetic analyses for comparison.

**Antimicrobial susceptibility testing**—*Salmonella enterica* isolates were tested for susceptibility to 16 antimicrobials. Minimum inhibitory concentrations of isolates were determined by use of broth microdilution in a semiautomated antimicrobial susceptibility testing system<sup>k</sup> in accordance with the manufacturer's instructions and guidelines published by the Clinical and Laboratory Standards Institute (formerly NCCLS). The panel of antimicrobial drugs used was established for use by the National Antimicrobial Resistance Monitoring System for enteric bacteria.<sup>11,12</sup> Isolates were categorized as susceptible, intermediate, or resistant using interpretive criteria published by Clinical and Laboratory Standards Institute, where available; otherwise, interpretive criteria used by the National Antimicrobial Resistance Monitoring System were used. *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as reference isolates for quality control of susceptibility testing.

**Statistical analysis**—The Dice coefficient was used to analyze the band patterns.<sup>1</sup> A dendrogram was constructed to

illustrate the genetic relatedness of the isolates; both genetic information and phenotypic information related to antimicrobial susceptibility were used in the evaluation.<sup>10,j</sup> Seventy percent and 90% were arbitrarily chosen as cutoff values to group genetically similar isolates for epidemiologic comparisons.

## Results

**Outbreak history**—The facility had functioned as a breeding operation for racing Greyhounds for approximately 5 years. An apparent increase in the morbidity and mortality rates had been recognized by the owners in several litters of 4- to 8-week-old puppies during the past year. Extensive health records were not available because many of the health assessments were performed only by the owner, who also initiated many of the treatments. The case definition used for this outbreak was 4- to 8-week-old Greyhound puppies that developed diarrhea or dysentery. In the 10 months prior to the investigation (ie, from October 2000 through August 2001), the owners reported 27 affected puppies from 8 litters, 10 (37%) of which died of illness consistent with this case definition. It is possible that this problem predated October of 2000, but that was the time at which the problem had first been recognized by the owners.

Examination of an epidemic curve and calculation of the morbidity and mortality rates revealed that there was often more than 1 ill puppy in affected litters but disease appeared to be sporadic among litters. It did not appear from available data that diarrheal illness and associated fatalities that had occurred in the past 10 months had an individual point source or propagating pattern among litters.

The clustered nature of illness within litters was consistent with contagious transmission in groups with close contact. The owners and referring veterinarian reported that affected puppies developed various clinical signs including fever, inappetance, lethargy, vomiting, diarrhea, dysentery, arthritis, and death. Specimens collected during necropsy of the 3 puppies that died prior to the site visit were cultured, and *S enterica* was isolated from the small intestine of 2 of those puppies. The referring veterinarian had also tested feces from several affected puppies for canine parvovirus, and results of those tests were negative. Sick puppies remained with their litters while being treated, and treatment protocols were essentially the same for all affected puppies. Sick animals were treated with fluids administered SC or IV depending on the degree of dehydration, and all affected puppies were treated with orally administered metronidazole<sup>l</sup> and sulfadimethoxine.<sup>m</sup>

**Dog population**—On the day of the site visit, 138 dogs resided at the premises. Owners reported that this was an open population, but that the number of dogs, as well as the age and use distributions of the population, had been stable during the previous year. Fifty-six adult Greyhounds were resident on the premises, including 47 breeding females, 3 breeding males, 4 aged adult females that were no longer used for breeding, and 2 adult males that were convalescing from racing injuries. There were also 82 puppies and juvenile dogs (< 1 year of age) at the facility. Of those dogs that were less than a year of age, 2 puppies were < 4 weeks

old, 19 puppies were  $\geq 4$  and < 8 weeks old, 9 puppies were  $\geq 8$  and < 16 weeks old, 12 puppies were  $\geq 16$  weeks and < 6 months old, and 40 juvenile dogs were  $\geq 6$  and < 12 months old. In addition, the owners kept 4 dogs of other breeds in their household on the premises; those dogs did not interact with the Greyhounds. Evidence of illness (eg, dysentery and somnolence) was noticed in only 1 puppy during the site visit; no abnormalities were observed in other dogs, and the feces of other dogs observed during the site visit had normal consistency.

**Housing**—The operation encompassed approximately 1.5 acres. Housing for Greyhounds was separated into 6 major areas according to dogs' age and use (Figure 1). Adult breeding animals and the youngest puppies were housed in a single building with concrete floors. Prior to whelping, breeding animals were housed in individual metal crates with wood floors (approx 80 × 80 × 110 cm) and exercised in small groups several times daily in outdoor enclosures with sand floors (approx 10 × 15 m). Pregnant females were moved just prior to their expected parturition date to larger indoor enclosures with concrete floors (approx 120 × 120 × 250 cm) where they remained until after whelping and puppies were 4 weeks old; females were allowed to exercise several times daily in separate outdoor enclosures. One- to 2-month-old puppies were housed with their dams in indoor pens (approx 120 × 120 cm) with attached outdoor runs with dirt floors (approx 2 × 10 m). After puppies were approximately 2 months old, they were weaned and housed in outdoor runs with small shelters until they were moved to a training facility at approximately 1 year of age. All 2- to 4-month-old puppies were grouped together in an outdoor enclosure (approx 10 × 15 m) that had a fully enclosed shelter with concrete flooring (approx 2 × 3 m). Four- to 6-month-old puppies were separated into groups of 3 to 6 dogs and housed in runs with compacted dirt floors (approx 2.5 × 15 m), each of which had a small (approx 1 × 2 × 2 m) shelter with a dirt floor. Groups tended to be comprised of puppies from a single litter, but this was not a strict rule. Juvenile 6- to 12-month-old dogs were moved to larger runs with compacted dirt floors (approx 4 × 65 m) that also had small (approx 1 × 2 × 2 m) shelters with dirt floors. Carpet remnants were provided as bedding for dogs throughout the facility. Carpet pieces were exchanged weekly and laundered between uses.

**Feeding and watering**—A custom ration was prepared on a daily basis and fed to all Greyhounds. Each day, 73 kg of coarsely ground raw beef was mixed with 41 kg of high-protein commercial kibble,<sup>n</sup> mineral and vitamin supplements, and garlic as a flavor enhancer. For 6- to 12-month-old dogs, raw tripe was substituted every other day for half of the raw beef. Raw meat products had reportedly been fed at the facility throughout the 5-year operating history. The owners did not maintain records related to purchasing or feeding of different batches of meat products. The ration containing raw meat was mixed each morning by hand as a single batch in a large bathtub installed in the kitchen area of the main building (Figure 1). Food was

placed in stainless steel bowls and provided ad libitum throughout the day in outside runs. Caretakers reported that there was usually some food left in bowls when they were collected in the morning, and at midafternoon on the day of the site visit, it was noticed that a large quantity of food remained in the bowls.

In the year prior to the investigation, raw meat fed to dogs had been purchased from a commercial slaughter and processing facility in Colorado (brand A) and more recently (10 weeks prior to the investigation), from a facility in Nebraska (brand B). Five of the 8 affected litters became ill while receiving brand A meat, and the remaining 3 affected litters became ill after switching to brand B meat. Both brand A and B products were composed entirely of beef that had been classified as unfit for human consumption by USDA inspectors, labeled accordingly, and marketed specifically as dog food. No instructions for cooking or other preparation of the meat product were printed on the packages. Beef was coarsely ground and sold frozen in boxes containing three 9.1-kg (20-lb) packages. All raw meat was delivered frozen and stored in a freezer adjacent to the main building. Each morning, meat that was to be fed the next day was set out and allowed to thaw at room temperature for about 24 hours. Dogs were provided fresh water ad libitum from a deep concrete-lined well that supplied water to the entire facility (including the owners' residence); water was provided to dogs in plastic bowls and buckets.

**Cleaning**—The owners and facility personnel made considerable efforts to clean animal housing areas throughout the day. There was relatively little animal or fecal odor at the premises at the time of the site visit, despite the number of animals on the premises and the stocking density. Hypochlorite solution (ie, bleach and water) was used to clean and disinfect surfaces in the main building (concentrations were not standardized). Floors in the main building were swept and mopped daily. Dog crates were moved every 2 weeks to facilitate cleaning of floors, and their interiors were cleaned monthly. Carpeting used as bedding was laundered and changed weekly. Hypochlorite solutions used in mop buckets were changed daily, and mop heads were changed every 1 to 2 months. Dog feces were removed at least daily from all areas of the facility and disposed of in a local landfill by a commercial garbage handling service. Cages and kennels inside the main building were cleaned before introduction of new animals, but no special cleaning or other precautions were taken when new groups of dogs were moved into outside enclosures. All food bowls and the kitchen tub and sink areas were cleaned daily with detergent and rinsed with hypochlorite solution, and bowls were stacked in the tub and sink to dry. Water buckets were scrubbed and rinsed with tap water weekly.

**Culture results**—Prior to the site visit, *S enterica* was recovered from puppies necropsied in October 2000 and July 2001. One hundred thirty-three samples were obtained during the site visit (Table 1), and of those, *S enterica* was recovered from 88 (66%). *Salmonella enterica* was recovered from 57 of 61 (93%) fecal samples and from 6 of 19 soil samples. Fecal sam-

ples with growth of *S enterica* were collected from all age groups of animals at the facility, and the soil samples containing *S enterica* were obtained from all outdoor housing and turnout areas, with the exception of the turnout area used for 2- to 4-month-old puppies. *Salmonella enterica* was recovered from 5 of 11 sponge samples obtained from floors in the kitchen and breeding and preweaning areas of the main building, from 1 of 2 sponge samples obtained from the kitchen sink, and from 3 of 8 sponge samples of cleaning tools. *Salmonella enterica* was cultured from the pool of flies that was captured in the kitchen area. *Salmonella enterica* was recovered from 6 of 8 brand B raw meat samples, 3 of 3 samples taken from food in bowls, and 6 of 10 samples taken from water buckets.

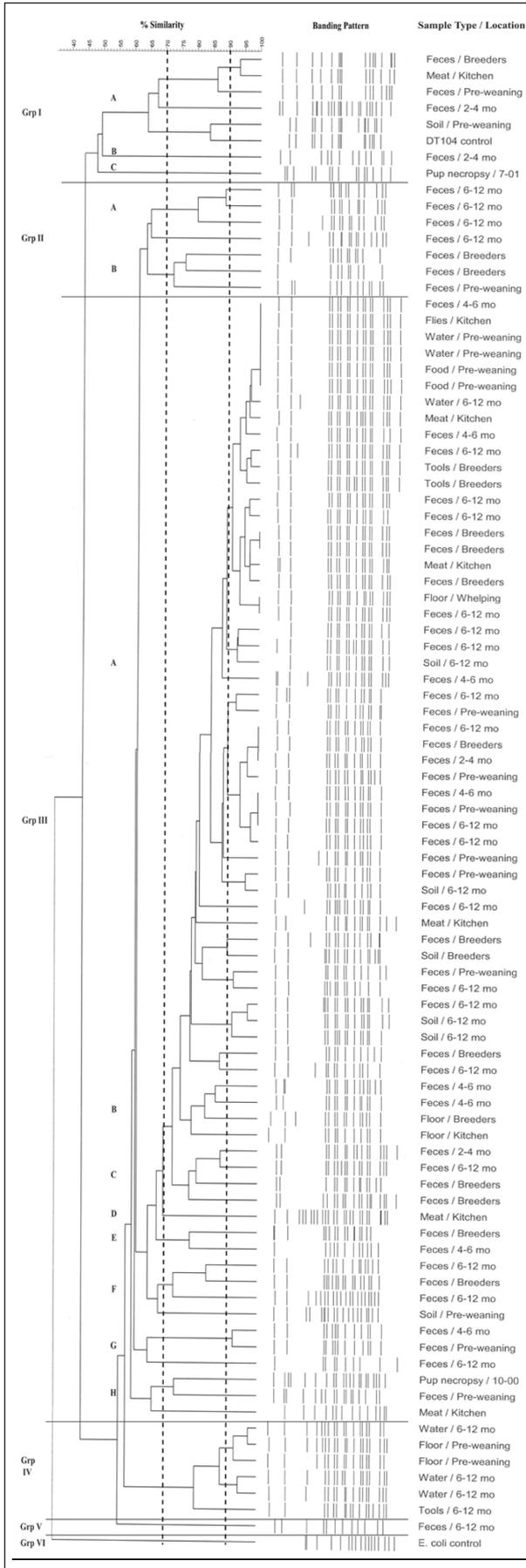
Of the 88 *S enterica* isolates recovered from samples collected during the site visit, 83 (94.3%) were serotype Newport, 3 (3.4%) were serotype Typhimurium, 1 (1.1%) was serotype Anatum, and 1 (1.1%) was serotype Uganda. The 5 Newport isolates were obtained from all areas of the facility. Two of the 5 Typhimurium isolates were recovered from fecal samples obtained in different housing areas, 1 *S typhimurium* isolate was recovered from the floor in the preweaning area of the main building, and the single *S Anatum* and *S Uganda* isolates were recovered from fecal samples. *Salmonella enterica* recovered from the puppy necropsied in October 2000 was serotype Newport, and the isolate recovered from the puppy necropsied in July 2001 was serotype Reading.

**Genetic analysis via PFGE**—The 88 *S enterica* isolates recovered from samples obtained during the site visit, 2 isolates recovered from necropsied puppies, and the reference strains for *S enterica* and *E coli* were separated into 6 genetically similar groups (arbitrarily designated I through VI) on the basis of results of PFGE analysis (Figure 2; Table 2). Isolates in PFGE groups with moderate to substantial genetic homology were further categorized into 15 subgroups (arbitrarily des-

Table 1—Summary of samples from which *Salmonella enterica* was isolated after collection during a site visit at a Greyhound breeding facility.

Sample type	No. of samples	Percentage (No.) from which <i>S enterica</i> was isolated
Feces	61	93 (57)*
Soil	19	32 (6)†
Brand A raw meat	8	0 (0)
Brand B raw meat	8	75 (6)†
Drippings from brand B raw meat	2	0 (0)
Food in bowls	3	100 (3)†
Sponges from inside water buckets	10	60 (6)†
Sponges of inside surfaces of kitchen sink	2	50 (1)†
Sponges of surfaces on cleaning tools	8	38 (3)†
Sponges of floor surfaces	11	45 (5)‡
Flies	1	100 (1)†
<b>Total</b>	<b>133</b>	<b>66 (88)</b>

\*Fifty-three isolates were serotype Newport, 2 were Typhimurium, 1 was Anatum, and 1 was Uganda. †All isolates were serotype Newport. ‡Four isolates were serotype Newport, and 1 was Typhimurium.



ignated A through H). With 90% homology used as a cutoff to assess clonality, 44 different clones were represented among the 88 isolates collected on the day of the site visit (1 group of 23 isolates had > 90% homology, 1 group of 10 isolates had > 90% homology, 3 groups of 3 isolates each had > 90% homology, 7 groups of 2 isolates each had > 90% homology, and the remaining 36 isolates had < 90% homology with any other isolate). With 70% homology used as a cutoff to assess clonality, 18 different clones were represented among the same 88 isolates (1 group of 56 isolates had > 70% genetic homology, 1 group of 6 isolates had > 70% homology, 3 groups of 3 isolates each had > 70% homology, 3 groups of 2 isolates each had > 70% homology, and the remaining 10 isolates had < 70% homology with any other isolate).

Pulsed-field gel electrophoresis group I was most similar to a reference strain of *S Typhimurium* DT104, whereas PFGE group VI was most similar to a reference strain of *E coli*. Isolates in PFGE groups II through V were all of serotype Newport with the exception of 1 *S Typhimurium* isolate that was recovered from the floor of the preweaning housing area. Group III included the largest group of isolates ( $n = 69$ ), including isolates from several highly similar clusters. Most (52/69 [75%]) of those isolates were assigned to subgroup III<sub>A</sub>. Notably, these highly similar isolates were distributed throughout the operation and were recovered from various types of sources including flies, feces, water, cleaning tools, food, and meat samples (Figure 2).

Although all 6 isolates recovered from brand B raw meat were of serotype Newport and had the same antimicrobial susceptibility pattern, there was considerable genetic diversity among them (Figure 2). Analysis of PFGE results suggested that the 6 isolates belonged to 4 different genetic clusters (PFGE subgroups I<sub>A</sub>, III<sub>A</sub>, III<sub>D</sub>, and III<sub>H</sub>). The 2 most closely related isolates from subgroups III<sub>A</sub> had > 90% genetic homology, the 3 isolates from III<sub>A</sub> had > 80% homology, and homology decreased substantially (< 65%, < 60%, and < 45%) when sequential comparisons were made that included isolates from the other 3 subgroups (III<sub>D</sub>, III<sub>H</sub>, and I<sub>A</sub>, respectively). A similar high degree of genetic diversity was observed in comparison of the 6 environmental isolates collected from the main building.

**Ribotyping**—The 88 *S enterica* isolates recovered from samples obtained during the site visit, the 2 isolates recovered from the necropsied puppies, and the reference strains for *S enterica* and *E coli* were categorized on the basis of results of ribotyping into 3 genetically similar groups (arbitrarily designated A1, A2, and B). Isolates in ribotype group A1 were genetically similar to *S Newport* isolates. Ribotype group A2 consisted of 6 isolates that were genetically similar to the *S Typhimurium* DT104 reference strain. Those isolates

Figure 2—Genetic relatedness of *Salmonella enterica* isolates recovered from field samples and necropsy specimens as determined by use of PFGE. Isolates are grouped by genetic similarity determined by computer analysis of PFGE banding patterns. Vertical lines connecting horizontal branches designate the estimated percentage of relatedness for a cluster of isolates, which can be read from the axis.

Table 2—Summary of genetic similarity as determined by use of PFGE, serotyping, source, and antimicrobial susceptibility pattern for 90 *S enterica* isolates recovered from field samples and necropsy specimens.

PFGE genetic group (n)	Percentage of genetic similarity within group	Serotypes (n)	Source* (n)	Resistance phenotypes
I (7)†	47.9	Anatum (1) Typhimurium (2) Newport (1) Reading (1) Uganda (1) Reading (1) Typhimurium reference (1) ‡	2–4 mo (2) Breeding (1) Prewaning (2) Kitchen (1) Necropsied puppy (1)	AMP, CMP, STR, SMO, TET (4); AMC, AMP, COX, CET, CTN, CMP, STR, SMO, TET (3)
II (7)	63.9	Newport (7)	6–12 mo (4) Breeding (2) Prewaning (1)	AMP, CMP, STR, SMO, TET (1); AMC, AMP, COX, CET, CTN, CMP, STR, SMO, TET (6)
III (69)	61.5	Typhimurium (1) Newport (68)	4–6 mo (8) Kitchen (7) Prewaning (13) 6–12 mo (23) Breeding (14) Whelping (1) 2–4 mo (2) Necropsied puppy (1)	AMP, CMP, STR, SMO, TET (2); AMC, AMP, COX, CET, CTN, CMP, STR, SMO, TET (66); AMC, AMP, COX, CET, CTN, CMP, GEN, KAN, STR, SMO, TET, TMS (1)
IV (6)	80.3	Newport (6)	6–12 mo (4) Prewaning (2)	AMP, CMP, STR, SMO, TET (1); AMP, CMP, KAN, NAL, STR, SMO, TET, TMS (1); AMC, AMP, COX, CET, CMP, STR, SMO, TET (1); AMC, AMP, COX, CRO, CTN, CMP, STR, SMO, TET (3)
V (1)	NA	Newport (1)	6–12 mo (1)	AMC, AMP, COX, CET, CTN, CMP, STR, SMO, TET (1)
VI (1)†	NA	<i>Escherichia coli</i> reference (1) ‡	NA	NA

\*Location on the premises from which sample was obtained (Figure 1). †Number includes reference strains. ‡Reference strain not isolated from facility.  
n = Number of isolates. AMP = Ampicillin. CMP = Chloramphenicol. STR = Streptomycin. SMO = Sulfamethoxazole. TET = Tetracycline. AMC = Amoxicillin-clavulanic acid. COX = Cefoxitin. CET = Ceftiofur. CTN = Cephalothin. GEN = Gentamicin. KAN = Kanamycin. TMS = Trimethoprim-sulfamethoxazole. NAL = Nalidixic acid. CRO = Ceftriaxone. NA = Not applicable.

were recovered from samples obtained from areas where breeding dogs were housed (floor sponges and feces) and from the areas where dogs were housed prior to weaning (soil and fecal samples). Ribotype group B included 1 *S Typhimurium* isolate that was genetically similar to the *E coli* reference strain and was recovered from the floor of a pen used to house dogs prior to weaning.

**Antimicrobial susceptibility**—Only 5 resistance phenotypes were identified among the 88 *S enterica* isolates recovered during the site visit and from the necropsied puppies (Tables 1 and 2). All isolates were resistant to chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, and all were susceptible to amikacin, apramycin, ceftriaxone, and ciproflaxacin. Seventy-nine of 90 (88%) isolates were resistant to 9 of 15 antimicrobial drugs evaluated (amoxicillin-clavulanate, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline). Of those 79 isolates, 77 (97%) were serotype Newport, 1 (1%) was serotype Uganda, and 1 was serotype Reading that was recovered from a necropsied puppy. Eight of 90

(9%) isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. Of those 8 isolates, 3 were serotype Typhimurium, 4 were serotype Newport, and 1 was serotype Anatum. Those 8 isolates were recovered from feces, floor and soil samples obtained in the preweaning area, and feces obtained from the breeding and 2- to 4-month-old dogs. One serotype Newport isolate recovered from a puppy necropsied in October 2000 was resistant to 12 antimicrobial drugs (amoxicillin-clavulanate, ampicillin, ceftiofur, cefoxitin, cephalothin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole).

## Discussion

Results of the investigation indicated that *S enterica* infections were common among all ages of dogs housed at the facility and that multiple clones across serotypes of *S enterica* were widely distributed in the environment. *Salmonella enterica* was cultured from raw meat fed to dogs, mixed food, and surfaces of water buckets. Food collected from bowls could have

been contaminated after preparation, but it is unlikely that raw meat was contaminated by exposure to something at the facility prior to sampling. Meat was sealed in plastic bags at the processing facility, sealed in cardboard boxes, and frozen prior to delivery to the kennel. Meat had been delivered frozen and was stored in a freezer until sampling; boxes and plastic bags had not been opened prior to sampling. Samples of the raw meat were taken directly from sealed packages and placed directly into sterile tubes for culture. Given the cross-sectional nature of this investigation, it was not possible to establish the temporality of exposures, but it appeared likely that at least some of the *Salmonella* strains recovered from the premises were introduced via the raw meat being fed.

Although recovery of *S enterica* from diets containing raw meat<sup>3,6,7</sup> and shedding of *S enterica* in dogs fed raw meat diets have been reported,<sup>5,8,9</sup> the present study links both *S enterica* infection and clinical salmonellosis in a population of dogs to feeding raw meat. This is an important finding because it has been proposed that dogs are not likely to be affected by pathogens contaminating raw meat diets, and the importance of raw meat diets as a source of *S enterica* and the health risks associated with exposing dogs to *S enterica* and other pathogens have been downplayed.<sup>13-18</sup> Recovery of *S enterica* from feces collected from a convenience sample of Greyhounds with diarrhea that were fed diets containing raw meat has been reported.<sup>8</sup> Although it was not clear from that report how many populations were sampled, how many dogs were affected, or how many dogs were exposed to the raw meat diets, those investigators reported a high degree of genetic relatedness among *S enterica* serotype Typhimurium isolates recovered from raw meat diets and feces, suggesting that raw meat was the source of exposure. In comparison, genotypic analysis of the *S enterica* isolates recovered during the present investigation indicated that multiple clones of *S enterica* had been introduced and disseminated in this population of dogs and that some fecal and environmental isolates were identical to isolates recovered from meat, indicating that meat was a probable source of the pathogen.

The high prevalence (93%) of *S enterica* shedding detected in this study is higher than shedding rates reported in 2 other studies<sup>3,8</sup> in which fecal shedding in diarrheic (63% and 76%) and nondiarrheic racing dogs (11% and 63%) that were fed raw meat diets was evaluated. These rates of *S enterica* shedding in racing dogs fed raw meat were considerably higher than rates of shedding among other populations of dogs that were not fed raw meat (0% to 2.8%), even when dogs had diarrhea.<sup>19-21</sup> Subjective comparison of these prevalences for *S enterica* shedding implicates the practice of feeding raw meat to dogs as a risk factor for *S enterica* infection in the dogs as well as indirectly suggesting an associated health risk to people in contact with the dogs.

A high degree of genetic diversity was observed among the *S enterica* isolates; there were 44 different isolates or groups with > 90% genetic homology and 18 different isolates or groups with > 70% homology. This finding suggests that the population was exposed to multiple strains and that extensive dissemination on

the premises was not solely attributable to propagation of a single clone. Recovery of 4 to 5 clones of *S enterica* from the raw beef fed on a single day illustrated that exposure to multiple strains could have been attributable solely to the contaminated raw meat that had been fed for several years. However, there were 2 groups of isolates with  $\geq 90\%$  within-cluster homology that represented 23 of 88 (26%) and 10 of 88 (11%) isolates, and 1 group with  $\geq 70\%$  within-cluster homology that represented 56 of 88 (64%) isolates. This clonal dissemination within the population could have resulted from exposure of the population to a single batch of heavily contaminated feed, or it could have resulted from propagation and spread within the population. The genetic analysis employed in the present study was an important adjunctive technique and illustrates the value of incorporating genetic comparisons in epidemiologic investigations of salmonellosis in populations. If this type of genetic analysis had not been used, it might have been assumed that all *S Newport* isolates were identical.

Contagious spread is a recognized feature of *S enterica* infections, and this was subjectively apparent in the history of clustering of clinical disease within litters. The pattern of disease reported among litters was best described as sporadic. However, this contrasted with the high rate of isolation (93% had positive culture results) of *S enterica* from fecal specimens on the day of the site visit, all but 1 of which were considered to be of normal consistency, in addition to the extensive environmental contamination (43% of samples had positive culture results). Further, all of the animals deemed ill were young puppies; no juvenile or adult dogs had been reported as ill. These findings suggest that immunity acquired through repeated exposures may have substantially influenced the occurrence of clinical disease. The cross-sectional nature of this investigation did not allow investigators to ascertain whether this level of exposure was consistent over time prior to the site visit. However, given the long-standing history of feeding raw meat, the recognized occurrence of disease over the previous 10 months, and the recovery of *S enterica* during this period, it seems possible that the rates of exposure and infection were not different during this site visit from rates that might have been observed in the past. It is possible that strains of *S enterica* that may have been introduced at other times had different inherent pathogenicity or immunogenicity, which may also have affected disease patterns over time. Dogs generally appeared to be in excellent physical condition, and no overt health problems were observed during the site visit. These observations were consistent with the history provided by the owner and the referring veterinarian and suggested that long-standing nutritional imbalances were not substantially affecting the health of the population over time. It is nevertheless possible that subtle imbalances associated with feeding an improperly balanced diet could have affected the dogs' immune function and physical abilities.

*Salmonella enterica* was not recovered from brand A raw meat, but 5 of the affected litters were being fed this product, and *S enterica* serotype Newport was recovered from a puppy that died in one of those litters. Investigators were only able to culture a limited

amount of brand A product, which had been stored in the freezer for several months, a much longer storage time than the brand B raw meat that was sampled. Viability of *S enterica* decreases when meat is stored frozen for extended periods of time, especially when frozen at warmer temperatures,<sup>22</sup> and long-term storage may have affected recovery of *S enterica* from meat in the present study. It is also possible that bacterial numbers were below detection limits. The extent to which the raw tripe may have contributed to prior exposures could not be evaluated because that product was not available for sampling on the day of the site visit. Given that the facility had problems with salmonellosis prior to switching brands of meat, it is plausible that brand A raw meat or the raw tripe were also contaminated with *S enterica*.

Several recommendations were made to the owners of the facility to control the exposure and spread of *S enterica*. The most highly prioritized recommendation was to feed only a high-quality, commercial, processed dog food or to thoroughly cook all meat prior to feeding. If the owners chose to continue feeding a customized ration, it was also strongly recommended that the diet be periodically analyzed and compared with National Research Council guidelines for canine diets. Although fecal material was collected frequently and disposed of, given the size and density of the dog population, it was recommended that dirt surfaces be replaced with a cleanable surface such as concrete. Frequent removal and disposal of fecal material in a manner that minimized subsequent exposure would continue to be important. It was recommended that cleaning protocols be adjusted to include scrubbing with a detergent prior to application of surface disinfectants. It was also recommended that areas be thoroughly cleaned and disinfected prior to moving dogs to new housing areas and that sick or newly introduced dogs be isolated before mixing with other dogs. Rigorous use of hand hygiene protocols and barrier nursing precautions are known to reduce transmission of infectious agents under various circumstances, and such protocols were recommended as essential in prevention of further illness in dogs and potential infection in human handlers. Rigorous control of insects and other pests was also encouraged because they may serve as reservoirs of *S enterica* in highly contaminated environments.<sup>23–25</sup> Approximately 10% of flies captured during a 1-year sampling period in a Greyhound kennel in which dogs were fed raw meat were found to be carrying *Salmonella* spp in 1 study.<sup>23</sup> Investigators are unaware of which recommendations were adopted following this investigation, but at the time this manuscript was prepared, the facility still reportedly had sporadic fatalities associated with enteritis in young dogs, and uncooked meat was still routinely being fed.

In addition to feeding practices, other husbandry factors likely contributed to the spread of *S enterica* throughout this facility. Commingling groups of dogs at a young age, failure to separate or isolate sick dogs or dogs newly introduced into the population, and limited use of hand hygiene procedures (washing or application of sanitizing solutions) after handling food or

feces or between handling dogs from different groups were all factors that may have contributed to the spread of different *Salmonella* strains at the facility. Additionally, thawing meat at room temperature for 18 to 24 hours and leaving the mixed ration in food bowls for 18 to 24 hours before removal may have allowed ample time for bacterial multiplication and increased the exposure doses for the dogs. This would be especially true during warm weather. Cooking of the meat prior to mixing or feeding a commercially prepared, cooked diet would have overcome this issue. Considering that all *S enterica* isolates recovered were resistant to sulfamethoxazole, regardless of serotype, treating sick dogs for short periods of time with a similar sulfa drug (sulfadimethoxine) may have contributed to selection of resistant strains that persisted in the population.

Many serotypes of *Salmonella* spp have been identified as causes of disease in humans and domestic animals, but most laboratory-confirmed *Salmonella* infections are attributed to a relatively small number of serotypes.<sup>26,27</sup> Typhimurium was the *S enterica* serotype most commonly isolated from clinically ill animals for many years.<sup>28</sup> However, isolation of multidrug resistant *S Newport* has increased substantially in prevalence throughout the United States during the past 6 years.<sup>10,28,29</sup> As reported by USDA–National Veterinary Services Laboratories, the number of *S Newport* isolates increased from 169 isolates in 1998 to 1,522 isolates in 2003.<sup>10,28,29</sup> The proportion of laboratory-confirmed *S Newport* infections in humans that were reported to the CDC increased from 5% (1,584/34,608) of all infections in 1997 to 11.5% (3,847/33,589) in 2003.<sup>12,19–28</sup> In 2003, *S Newport* was the second most common isolate obtained from animals without clinical signs of infection ( $n = 888$ ; 16.6% of all nonclinical isolates from animals), the sixth most common isolate from clinically affected animals ( $n = 290$ ; 5.1% of all clinical isolates from animals), and the third most common serotype from clinically affected humans ( $n = 3,847$ ; 11.5% of all human isolates).<sup>28</sup> *Salmonella enterica* serotype *Newport* can be associated with high morbidity and mortality rates in infected animals, and many dairy cattle that are infected are culled as a result of poor milk production, poor condition, and clinical illness.<sup>29</sup> Studies<sup>30,31</sup> of dairy cows just prior to culling and at the time of slaughter suggest that *S enterica* can be recovered from 18% to 25% of cows from a single culture of feces or colon contents. If lesions were found in these animals at slaughter, meat would be labeled unfit for human consumption, as were the raw meat products fed to dogs at the facility in the present study. This labeling does not prohibit the legal sale of such products as food for pets or other animals,<sup>2</sup> and these raw meat diets have been a source of exposure to *Salmonella* spp in dogs.<sup>3–9</sup>

The reported rates (6% to 73%)<sup>3,6–8</sup> of isolation of *S enterica* from raw meat and mixed diets containing raw meat that were sold for consumption by pets are consistent with the high rate of isolation observed in the present investigation (9/19). However, it is important to note that all food products have the potential to be contaminated with enteric pathogens, and even

meat sold for human consumption may be contaminated with *S enterica*. Surveys of retail meat products conducted by the FDA in 2002 and 2003 revealed that *S enterica* could be cultured from approximately 1% of ground beef samples, compared with approximately 1% of pork chop samples, 9.5% of chicken breast samples, and 12% of ground turkey samples.<sup>11</sup> Those reports strongly support the recommendation to feed appropriately cooked diets to pets to prevent infection with foodborne pathogens and subsequent exposure risk to humans.

- a. Hydra-Sponge, Biotrace International, Bothell, Wash.
- b. Tetrathionate medium, BD Difco, Sparks, Md.
- c. XLT-4 agar, Hardy Diagnostics, Santa Maria, Calif.
- d. Trypticase soy agar, BD BBL, Sparks, Md.
- e. Seakem Gold, BioWhittaker Molecular Applications, Rockland, Me.
- f. *Xbal*, Promega, Madison, Wis.
- g. CHEF mapper pulsed-field electrophoresis system, Bio-Rad Laboratories, Hercules, Calif.
- h. Tris-borate-EDTA, TBE, Sigma-Aldrich Inc, St Louis, Mo.
- i. Bionumerics, Applied Maths Scientific Software Development, Sint Martens Latern, Belgium.
- j. Sensititre, Trek Diagnostic Systems, Westlake, Ohio.
- k. Riboprinter, DePont Qualicon, Wilmington, Del.
- l. Flagyl, Pfizer, New York, NY.
- m. Albon, Pfizer Animal Health, New York, NY.
- n. Purina Hi-Pro, Nestle Purina, St Louis, Mo.

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