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Prevalence of *Salmonella* in raw meat used in diets of racing greyhounds

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Abstract. One hundred twelve samples of commercial raw meat used in greyhound diets were collected and cultured for *Salmonella* using standard procedures. Fifty (44.64%) of these samples were positive for *Salmonella. Salmonella typhimurium* was the most frequently isolated serovar (48%), followed by *S. newport* (12.76%), S. *agona* (8.51%), and *S. muenster* (6.38%). The remaining 10 serovars recovered in this study represented 27.59% of the total *Salmonella* isolates. In addition, the meat samples were screened for *Salmonella* using a commercial DNA probe. Of the 106 samples tested, 70 (66.03%) were positive for *Salmonella,* which indicated that the DNA probe assay was more sensitive than the culture method for screening of *Salmonella* in raw meat. Antimicrobial susceptibility testing revealed that most of the *Salmonella* isolates were sensitive to a variety of antimicrobials, particularly amikacin and apramycin, and resistant to some others, such as clindamycin, erythromycin, penicillin, and sulfadimethoxine. The cumulative percentages of susceptibility (MIC,, and MIC,,) of the *Salmonella* isolates were also determined. Most isolates were susceptible (MIC,,) to low concentrations of gentamicin (2.0 &ml), imipenem (≤0.25 μg/ml), and ciprofloxacin (≤0.5 μg/ml). Marked resistance was found with the other antimicrobial agents. However, the high MIC values found for these isolates would not be achievable in vivo with the normal recommended doses of antimicrobial agents, so their use would not be beneficial. Numerous plasmid patterns were found in 17 randomly selected *Salmonella* isolates. Eight of the 17 isolates had 2-7 plasmids ranging from 2.4 to 15 kilobases in size. Eight isolates also exhibited large plasmids in the range of 50-60 and 95-105 kilobases. Large plasmids migrated above the chromosal DNA. Six isolates did not demonstrate any visible plasmids.

Salmonellae are natural inhabitants of the intestinal tract of domestic and wild animals of all types and are shed by infected animals in fecal material and often in urine [11] From these sources, the bacteria contaminate the environment and can grow in food and water and on inanimate objects. Salmonellae grow well at room temperatures (24-26 C) and thus are able to increase in numbers in contaminated foods. Processing provides opportunity for food to be contaminated from the environment, from a worker carrying the organism, or from a worker infected by the organism. According to numbers of cases reported and the opinions of many public health officials, salmonellosis is one of the most common infectious diseases transmitted by contaminated foods. [11]

Raw meat from rendering plants constitutes 50-75% of the diet of racing greyhounds (N. H. Gabbert, personal observation). The meat comes from diseased, debilitated, dying, and dead animals (4-D meat) and can contain pathogenic organisms, including *Salmonella, Campylobacter jejuni,* and *Escherichia coli* (M. M. Chengappa, personal observation). The meat is ground and frozen in 5-, 8-, 25-, or 40-pound (2.3., 3.6-, 11.4., or 18.2-kg) packages containing meat from multiple carcasses. At the kennel, the meat is thawed at room temperature for 24 hr and then mixed with other feeds and feed supplements. Kennel personnel frequently mix dog food with bare hands, and cleanliness is not easily maintained or possible. Cooked meat is not routinely used because dogs are believed to perform better on raw meat (N. H. Gabber-t, personal observation).

Salmonellosis generally is common in dogs and almost always occurs as a result of ingestion of contaminated food.[3] *Salmonella* infections in sentry dogs have been attributed to raw horse meat[2] and to diets prepared in veterinary hospitals.[7] *Salmonella* organisms have been found in more than half the samples from slaughter houses processed for human consumption.[21] Of the 24 serotypes recovered from the samples, *Salmonella typhimurium* was the most prevalent. The number of dogs infected with salmonellae is surprisingly high and is greater than the incidence of clinical disease would suggest.[9] Thus, there is both economic and public health significance to the problem of salmonellosis.

Outbreaks of *Salmonella* enteritis ("kennel sickness," "blowout") and of systemic salmonellosis are common among greyhounds in kennels.[3,7,22] During such outbreaks, *Salmonella* can be found in the 4-D meat as well as in feces. Isolation of *Salmonella* from

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the feces of clinically healthy or hospitalized pet dogs has been reported to range from 1% to 36%.[12,19] The *Salmonella* infections appear to be associated with clinical signs and pathologic evidence of canine distemper. Salmonellosis is frequently responsible for concurrent viral enteritis and vaccine failures in dogs. The mechanisms behind this apparent association are not clear; however, immunosuppression is believed to play a significant ro1e.[9] The dual infection becomes a diagnostic, therapeutic, and prognostic nightmare.

The purpose of this research was to determine the prevalence of *Salmonella* in raw meat from rendering plants that is used in racing greyhound diets and to generate antibiograms and plasmid profiles of the *Salmonella* isolates recovered. Once the prevalence of *Salmonella* is established, appropriate steps can be taken to minimize the presence of these organisms in such raw meat.

Materials and methods

Raw meat. Commercial raw meat products were purchased from several major companies. Five packages of each product were purchased during the months of January, February, April, May, July, September, October, and November for *Salmonella* screening. Meat product was frozen when purchased and stored at - 15 C until cultured.

Cultures. From each package, 25 g of meat was placed in 225 ml of lactose broth[a] and incubated for 16-18 hr at 37 C. Following incubation, 1 ml of lactose broth culture was inoculated into a tube containing 10 ml of tetrathionate broth,[a] incubated overnight at 37 C, then streaked onto Hektoen enteric agar[a] and MacConkey agar[a] plates. The plates were incubated at 37 C for 18 hr, then all *Salmonella* suspects were characterized biochemically and serologically by standard procedures.[3] Further serologic characterization was done at the National Veterinary Service Laboratory, Ames, Iowa. Additional tests were performed with the meat samples using genus-specific DNA probes as described by the manufacturer.[b]

Antimicrobial susceptibility testing. Two methods were employed: qualitative dilution and quantitative dilution. Both were done using a commercial antimicrobial susceptibility testing system.[c] The antimicrobial agents and their concentrations are presented in Tables 1 and 2. Results of the qualitative test were expressed as susceptible, moderately susceptible, and resistant. The quantitative test provided the minimal inhibitory concentration (MIC) of the drugs expressed in micrograms per milliliter. The MIC is defined as the lowest concentration of a drug that inhibited visible bacterial growth and measures the bacteriostatic or bactericidal effect of the antimicrobial agent.

Plasmid analysis. Salmonella isolates used in this study were selected randomly. These isolates had various degrees of resistance to the 18 antimicrobial agents tested (Table 3). Total bacterial DNA (both chromosomal and plasmid) was extracted according to a recently described procedure.[6] *Salmonella* isolates that had been cultured from raw meat used in diet formulation for racing greyhounds were grown in 2 ml of brain-heart infusion broth[a] overnight at 37 C. The cells were harvested by centrifugation at 5,000 x g for 20 min at 4 C. The resulting pellet was transferred to a microcentrifuge tube and stored at -80 C until used.

Table 1. Antimicrobial susceptibility testing results of 50 *Salmonella* isolates as determined by the qualitative method.

Antimicrobial agent	Agent concentration (μg/ml)	% resistant	% susceptible	% moderately susceptible
Amikacin	16, 32	0	100	0
Ampicillin	2, 4	56	42	2
Apramycin	16	0	100	0
Augmentin*	4/2, 16/8	6	73	21
Carbenicillin	16, 64	33	63	4
Ceftiofur	1, 2	92	6	2
Cephalothin	8, 16	8	88	4
Chloramphenicol	8, 16	35	73	2
Clindamycin	0.5, 4	100	0	0
Enrofloxacin	1, 4	4	96	0
Erythromycin	0.5, 4	100	0	0
Gentamicin	4, 8	6	92	2
Neomycin	8	54	46	0
Oxacillin	2, 4	100	0	0
Penicillin G	0.12, 2	100	0	0
Sulfadimethoxine	20, 40	100	0	0
Tetracycline	4, 8	88	12	0
Timentin†	16/2, 64/2	21	64	15
Trim/Sulfa‡	1/19, 2/38	25	75	0

* Amoxicillin/clavulanic acid.
† Ticarcillin/clavulanic acid.
‡ Trimethoprim/sulfamethoxazole.

After thawing, the sample was resuspended in 500 μl of TE buffer (10 mM Tris HCl, pH 7.4; 1 mM ethylenediaminetetraacetic acid [EDTA]), transferred to a microcentrifuge tube containing 400 μl of a 1:1 mixture of phenol : chloroform, shaken vigorously for 20 sec, held at -20 C for 30 min, and centrifuged at 12,000 x g for 15 min at 4 C. The aqueous phase was transferred to another tube, and the phenol : chloroform step was repeated. DNA was precipitated by adding 0.1 volume of 3 M sodium acetate and 1 volume of isopropanol, washed twice with 70% ethanol, dried under vacuum, and dissolved in TE buffer (10 mM Tris HCl, pH 7.0, 1 mM EDTA).

The total bacterial DNA was electrophoresed on a 0.8% agarose gel to separate plasmid from chromosomal DNA. The plasmid DNA was recovered by electroelution. Purified plasmid DNA preparations were electrophoresed in 1% agarose gels at 2 V/cm for 16 hr in TBE buffer (20 x TBE = 1 M Tris base, 1 M boric acid, 20 mM EDTA). Following electrophoresis, the gels were stained with ethidium bromide (0.25 μg/ml) for 45 min and photographed with UV light illumination (Polaroid #667). Supercoiled DNA ladders (16.2 10-2.067 kilobases [kb]) were used as molecular size markers[d] for plasmids in the size range of 2.4-15 kb. For larger sizes, plasmids of known size from *Salmonella typhimurium* (ATCC 13311 [95-101 kb]), *Salmonella dublin* (ATCC 15480 [50 kb], *Salmonella choleraesuis* var. *kunzen-*

Table 2. Antimicrobial minimal inhibitory concentration (MIC) of 50 Salmonella isolates recovered from greyhound diets.

Antimicrobial agents	Dilution range (μg/ml)	MIC interpretive standards*			MIC_{50}†	MIC_{90}†
		Susceptible	Moderately susceptible	Resistant		
Ampicillin	0.12–16.0	≤1.0	2.0–8.0	≥16	8	16
Cephalothin	0.5–64.0	≤4.0	8.0–32.0	≥64	4	32
Chloramphenicol	4.0–32.0	≤4.0	8.0–16.0	≥32	8	32
Ciprofloxacin	0.5–4.0	≤0.5	1.0–2.0	≥4	≤0.5	≤0.5
Clindamycin	0.12–16.0	≤1.0	2.0–8.0	≥16	≥16	≥16
Erythromycin	0.12–16.0	≤1.0	2.0–8.0	≥16	≥16	≥16
Gentamicin	0.25–32.0	≤2.0	4.0–16.0	≥32	0.25	2
Imipenem	0.25–32.0	≤2.0	4.0–16.0	≥32	≤0.25	≤0.25
Oxacillin	0.25–32.0	≤2.0	4.0–16.0	≥32	≥32	≥32
Penicillin G	0.03–4.0	≤0.25	0.5–2.0	≥4	≥4	≥4
Rifampicin	0.5–4.0	≤0.5	1.0–2.0	≥4	≥4	≥4
Sulfamethoxozol	4.75–76.0	≤4.75	9.5–38.0	≥76	≤4.75	76
Tetracycline	2.0–16.0	≤2.0	4.0–8.0	≥16	≥16	≥16
Trimethoprim	0.25–4.0	≤0.25	0.5–2.0	≥4	≤0.25	4
Vencomycin	0.5–64.0	≤4.0	8.0–32.0	≥64	≥64	>64

* Adapted from the National Committee on Clinical Laboratory Standards, provided by manufacturer.
† The concentration at which 50% and 90% of the isolates were found susceptible.

dorf (ATCC 12011 [80 kb]), PIG 995 (29 kb), and λ phage (19 kb) were used as standard markers.

Results

One hundred twelve commercial raw meat samples were collected and cultured for *Salmonella* using standard procedures. Collection periods and numbers of samples collected are listed in Table 4. Fifty (44.64%) of these samples were positive for *Salmonella* by culture. All 50 *Salmonella* isolates recovered from the samples by culture techniques were serotyped using commercially prepared antisera. Serovar distribution of these isolates is presented in Table 5. *Salmonella typhimurium* was the most frequently isolated serovar (48%), followed by *S. newport* (12.76%), *S. agona* (8.51%), and *S. muenster (6.38%).* The remaining 10 serovars were less frequently recovered and represented 27.59% of the total *Salmonella* isolates recovered. Of the 106 samples tested using the commercial DNA probe, 70 (66.03%) were positive for *Salmonella.*

Antimicrobial susceptibility testing revealed that most of the *Salmonella* isolates were sensitive to amikacin (100%), apramycin (100%), augmentin (73%), carbenicillin (63%), cephalothin (88%), chloramphenicol (73%), enrofloxacin (96%), gentamicin (92%), timentin (64%), and trimethoprim/sulfamethaxozole (75%), and resistant to ceftiofur (92%), clindamycin (100%), erythromycin (100%), penicillins (100%), sulfadimethoxine (100%), and tetracycline (88%) (Table 1)

The cumulative percentages of susceptibility (MIC_{50} and MIC_{90}) of the *Salmonella* isolates are presented in Table 2. Most isolates were susceptible to low concentrations (MIC_{90} of gentamicin (2.0 μg/ml), imipenem (≤0.25 μg/ml), and ciprofloxacin (≤0.5 μg/ml). Marked resistance was found with the remaining antimicrobial agents. The high MIC values found with these isolates would not be achievable in vivo with normal recommended doses of antimicrobial agents, so their use would not be beneficial.

Numerous plasmid patterns were found in the 17 randomly selected *Salmonella* isolates. Their serovar designations and antibiograms are presented in Table 3. Electrophoresis of plasmid preparations revealed heterogenous profiles (Figs. 1, 2). Eight strains had 2-7 plasmids ranging from 2.4 to 15 kb in size. Isolates 9, 11, and 16-20 contained plasmids migrating in the 95-105-kb range. Plasmids in the range of 50-60 kb were also identified with some frequency in isolates 4 and 17-19. These plasmids seemed to be present in higher copy number than the 95-105-kb plasmids. Also identified were plasmids of approximately 30 kb in isolates 11 and 20 migrating above the chromosomal DNA and a single plasmid in isolate 13 migrating above the 29-kb marker and below the 50-kb marker.

Discussion

Salmonellosis in greyhounds is common[3] and affects higher numbers of dogs than the incidence of clinical disease would suggest.[9] Morbidity in greyhound puppies from salmonellosis can approach 100%, and mortality ranges to nearly 40% (N. H. Gabbert, personal observation). The economic impact of these losses to the industry is obvious. The additional economic loss from poor growth and performance caused by salmonellosis is not easy to document, but it is also very significant. Infected dogs often become carriers and may be public health hazards to those humans in con-

Table 3. Cumulative antimicrobial profiles of 17 Salmonella isolates randomly selected for plasmid analysis.

Serovar	% resistant	% susceptible	% moderately susceptible
S. agona	68	21	11
S. anatum	37	63	0
S. dublin	58	42	0
S. enteritidis	52	37	11
S. enteritidis	32	68	0
S. muenster	32	68	0
S. newport	63	26	6
S. newport	47	42	11
S. newport	37	63	0
S. newport	47	42	11
S. reading	32	68	0
S. schwarzengrund	37	63	0
S. typhimurium	57	37	6
S. typhimurium	57	37	6
S. typhimurium	57	32	11
S. typhimurium	32	68	0
S. typhimurium biovar *Copenhagen*	52	37	11

tact with them. Further, infection with *Salmonella* causes immunosuppression in animals that may lead to concurrent bacterial or viral diseases.[9]

Racing greyhounds contract salmonellosis primarily by eating contaminated 4-D meat. The present study indicated that 44.64% and 66.03% of raw meat samples were positive for *Salmonella* by culture and DNA probe methods, respectively. Although the DNA probe method is more sensitive than the conventional culture method, the results of the culture method were more useful because they provided serovar designation and allowed antimicrobial susceptibility profiles of all *Salmonella* isolates. The conventional methods used at present by most diagnostic laboratories for isolation and identification of salmonellae in food products are somewhat time consuming. The probe assay is less time consuming, more sensitive, and easy to perform with minimum training but may be less suitable for

Table 4. Results of *Salmonella* testing of raw meat diet collected in various months during 1990-1991.

Collection period	No. samples collected	No. samples positive/no. tested	
		Culture	DNA probe
October 1990	15	9/15 (60%)*	10/15 (67%)
November 1990	16	8/16 (50%)	8/12 (67%)
January 1991	14	6/14 (43%)	6/12 (50%)
February 1991	15	9/15 (56%)	10/15 (67%)
April 1991	13	2/13 (15%)	6/13 (46%)
May 1991	12	6/12 (50%)	10/12 (83%)
July 1991	13	8/13 (62%)	9/13 (69%)
September 1991	14	2/14 (14%)	11/14 (79%)

* Percent positive.

Table 5. Serovars of *Salmonella* recovered from raw meat diet by culture technique.

Serovars	No. samples positive	% positive
S. typhimurium	24	48.00
S. newport	6	12.76
S. agona	4	8.51
S. muenster	3	6.38
S. anatum	2	4.25
S. enteritidis	2	4.25
S. schwarzengrund	2	4.25
S. bardo	1	2.12
S. dublin	1	2.12
S. mbandaka	1	2.12
S. reading	1	2.12
S. senftenberg	1	2.12
S. thomasville	1	2.12
S. worthington	1	2.12

many diagnostic laboratories for economic reasons. DNA-based diagnostic kits can also be extremely useful for detecting the presence of other microorganisms. DNA probes against several microorganisms could be used simultaneously to detect pathogens that might be present in fecal samples of animals or humans with gastroenteritis.

Because salmonellosis is almost always caused by ingestion of contaminated material, much interest has been directed toward the identification of sources of infection. *Salmonella typhimurium* has been the most frequently identified serovar from outbreaks of diarrheal diseases in humans and animals.[5,14,15,17,18,24] Because *S. typhimurium* was the most frequently recovered serovar from the 4-D meat samples (Table 4), and feeding greyhounds this type of meat is a common practice, *S. typhimurium* is probably the predominant serovar involved in any diarrheal diseases occurring in greyhounds following feeding of this meat. Dogs are a potential source of human salmonellosis; puppies are responsible for most clinical cases of human salmonellosis.[15] The source of *Salmonella* in those cases was feces.

Improved awareness of the meat contamination problem is essential because the quality and safety of greyhound diets are vital for the well-being of the industry. The microbiologic quality of greyhound diets can have major implications for their deterioration during storage, their nutritional value, and their effect on greyhound health and performance. There is clearly room for improvement in the quality management and manufacturing practices of the meat industry for greyhounds. The dog owners and other kennel personnel should be made aware of the dangers involved in feeding 4-D meat products. Preventive measures such as proper cooking, chemical treatment, and proper han-

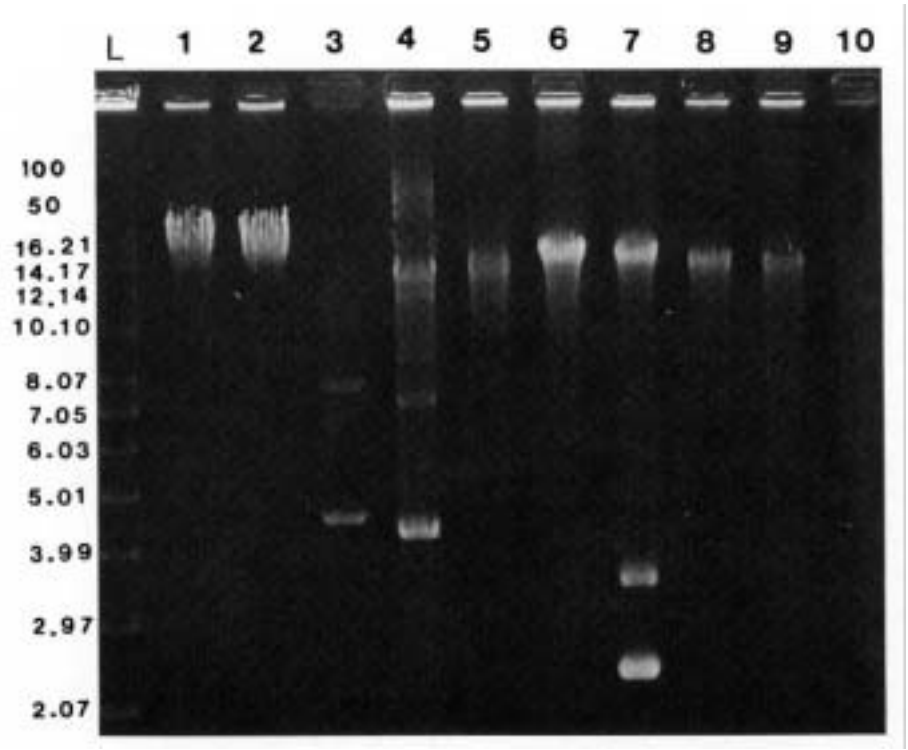

Figure 1. Plasmid patterns of *Salmonella* isolates. Lane L: supercoiled DNA size standards; lanes 1, 2: total chromosomal DNA of S. *reading* and *S. agona,* respectively; lane 3: *S. muenster;* lanes 4, 9: *S. reading* (same isolate, but maintained at 2 different temperatures, -60 C [lane 41 and 25 C [lane 9]); lane 5: *S. newport;* lane 6: *S. dublin;* lane 7: *S. typhimurium;* lane 8: *S. enteritidis;* lane 10: *S. agona.*

dling and storage of meat should also be brought to the attention of people in the industry.

The increase in resistance of *Salmonella* to antimicrobial agents has been a major problem for practitioners of veterinary and human medicine. [1] Plasmids of all sizes have been implicated in the virulence and resistance to antimicrobial agents of *Salmonella* isolates.[4,10,20] Some *Salmonella* serovars are known to harbor plasmids in the 50-100-kb range, which are virulence plasmids and include serovar-specific plasmids

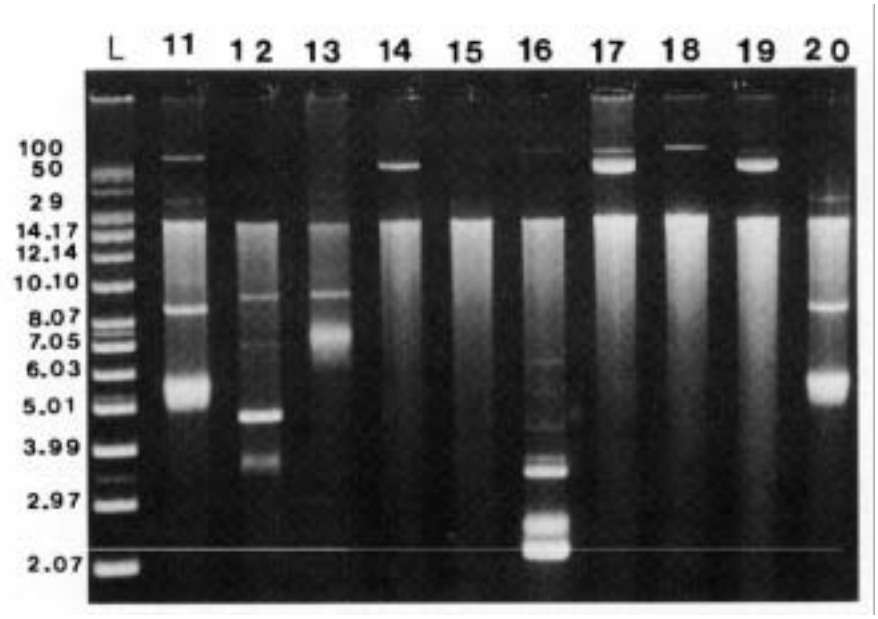

Figure 2. Plasmid patterns of *Salmonella* isolates. Lane L: supercoiled DNA size standards; lanes 11, 12, 16: *S. typhimurium* (3 different isolates); lane 13: *S. anatum;* lane 14: *S. enteritidis;* lane 15: *S. schwarzengrund,* lanes 17-19: *S. newport (3* different isolates); lane 20: *S. typhimurium* biovar *Copenhagen.*

from *S. typhimurium, S. dublin,* and *S. enteritidis* of 100, 83, and 60 kb, respectively.[13,16,23] Large plasmids in the 65 megadalton range (approximately 100 kb) confer tetracycline resistance in *Salmonella.*[8] Results of the present study indicate the presence of numerous plasmids ranging from 2.4 to 105 kb in size (Figs. 1, 2). The association of these plasmids with certain virulence and drug resistance characteristics should be investigated. The potential problem of spread of these plasmid-carrying *Salmonella* to other animals and humans is serious. Individuals directly associated with kennel operations are placed in a hazardous situation.

These data on isolation of *Salmonella* from raw meat, the resistance of *Salmonella* to various antimicrobial agents, and the potential spreading of *Salmonella to* healthy animal and human populations show that efforts are needed in the greyhound industry to improve the control of *Salmonella* contamination in raw meat used in diets. This study emphasizes the need for more detailed research and discussion concerning the role feeding of 4-D meat to greyhounds plays in the epidemiology of salmonellosis. Increased awareness of the problem and surveillance programs concerning the quality and safety of greyhound diets are important areas for future efforts.

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Sources and manufacturers

a. Difco Laboratories, Detroit, MI.
b. Integrated Genetics, Framingham, MA.
c. Sensititre Microbiology Systems, Westlake, OH.
d. Bethesda Research Laboratories, Gathersburg, MD.

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