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Surveillance of *Salmonella* Prevalence in Animal Feeds and Characterization of the *Salmonella* Isolates by Serotyping and Antimicrobial Susceptibility

X. Li,[1] L.A. Bethune,[2] Y. Jia,[3] R.A. Lovell,[1] T.A. Proescholdt,[1] S.A. Benz,[1] T.C. Schell,[1] G. Kaplan,[1] and D.G. McChesney[1]

Abstract

This article presents the surveillance data from the Feed Contaminants Program (2002–2009) and *Salmonella* Assignment (2007–2009) of the U.S. Food and Drug Administration (FDA), which monitor the trend of *Salmonella* contamination in animal feeds. A total of 2,058 samples were collected from complete animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets in 2002–2009. These samples were tested for the presence of *Salmonella*. Those that were positive for *Salmonella* underwent serotyping and testing for antimicrobial susceptibility. Of the 2,058 samples, 257 were positive for *Salmonella* (12.5%). The results indicate a significant overall *Salmonella* reduction ($p \leq 0.05$) in animal feeds from 18.2% (187 samples tested) in 2002 to 8.0% (584 samples tested) in 2009. Among these samples, feed ingredients and pet foods/treats had the most significant reduction ($p \leq 0.05$). Of the 45 *Salmonella* serotypes identified, *Salmonella* Senftenberg and *Salmonella* Montevideo were the top two common serotypes (8.9%). Of the 257 *Salmonella* isolates obtained, 54 isolates (21%) were resistant to at least one antimicrobial. The findings provide the animal feed industries with *Salmonella* prevalence information that can be used to address *Salmonella* contamination problems. Our findings can also be used to educate pet owners when handling pet foods and treats at home to prevent salmonellosis.

Introduction

Salmonella is the most common foodborne pathogen in the United States, causing approximately 17.6 illnesses per 100,000 persons, 2,290 hospitalizations, and 29 deaths in 2010 (Scallan *et al*., 2011). No significant change in incidence of *Salmonella* infection in humans has occurred since the start of surveillance during 1996–1998 (CDC, 2011). Currently, there are more than 2,500 known serotypes of *Salmonella* (Popoff *et al*., 2003).

Salmonella infection in humans can be traced back to handling or consuming contaminated foods, such as those of animal origin (Zhao *et al*., 2009). *Salmonella* can also be spread directly to humans by handling contaminated pet foods and pet treats. The association between human outbreaks of salmonellosis and contact with *Salmonella*-contaminated pet foods and pet treats is well established. Notably, Canadian outbreaks of human salmonellosis were linked to *Salmonella* Infantis in pig-ear dog treats that were manufactured in Canada in 1999, and to *Salmonella* Newport in beefsteak-patty dog treats that were manufactured in Texas in 2002 (Health Canada, 2000; Clark *et al*., 2001; Pitout *et al*., 2003). In addition, human outbreaks of salmonellosis in western Canada and Washington State in the United States in 2004–2005 were linked to pet treats contaminated with *Salmonella* Thompson (Health Canada, 2006). More recently, the Centers for Disease Control and Prevention (CDC) reported that, between January 2006 and December 2007, 70 human cases of salmonellosis were linked to *Salmonella* Schwarzengrund in dry dog foods that were manufactured by a company in the United States (CDC, 2008).

Humans becoming infected with *Salmonella* through contact with pet foods and pet treats has become an increasing concern because household pets are extremely common in the United States. In 2002, it was estimated that 39% of households had a dog and 34% had a cat (Finley *et al*., 2006). The FDA has termed this direct route of exposure from handling animal feeds, pet foods, and pet treats as exposure from "Direct-Human-Contact Feeds." This category includes animal feeds that are intended for use in feeding animals in

[1]Office of Surveillance and Compliance, Center for Veterinary Medicine, U.S. Food and Drug Administration, Rockville, Maryland.
[2]Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts.
[3]Office of Biotechnology Products, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland.

homes, petting zoos, agricultural fairs, and similar venues where they are likely to be directly handled or ingested by humans. *Salmonella*-contaminated Direct-Human-Contact Feeds pose a significant health risk to humans. Certain vulnerable populations such as children, the elderly, and individuals with compromised immune systems, are particularly susceptible to *Salmonella* infections from such feeds (FDA, 2009).

Follow-up investigations of *Salmonella* outbreaks in the United States and Canada found that pet foods and treats are frequently contaminated with *Salmonella* (Health Canada, 2006). For example, following the 1999 outbreak of *Salmonella* Infantis that was attributed to pig-ear dog treats, *Salmonella* was isolated from 51% of pig-ear dog treats sampled from retail stores in Alberta, Canada, and 41% of dog treats sampled in retail stores in the United States (Health Canada, 2006).

To monitor the trend of *Salmonella* contamination in animal feeds, since 2002, the FDA Center for Veterinary Medicine (CVM) has established a *Salmonella* surveillance program under the Feed Contaminants Program to collect samples from complete animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets in domestic commerce and at the Untied States Ports of Entry. Subsequently, since 2007, the FDA CVM has established a second *Salmonella* surveillance program (*Salmonella* Assignment) to focus sample collection on Direct-Human-Contact Feeds that include complete finished animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets in the United States. This article presents 2002–2009 surveillance data from both programs and discusses the trend of *Salmonella* prevalence in animal feeds.

Methods

Sample collection and sampling size

Samples collected under the FDA CVM Feed Contaminants Program (2002–2009) were from complete animal feeds (finished feeds), feed ingredients, pet foods, pet treats, and supplements for pets in domestic commerce and entering the United States through Ports of Entry. In addition, samples of Direct-Human-Contact Feeds (complete finished animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets) collected under the *Salmonella* Assignment (2007–2009) were collected from manufacturers, distributors, wholesalers, or retailers (including pet stores, petting zoos, agricultural fairs, and festivals) in the United States. All samples were collected by using aseptic technique. Each sample consisted of 10 subsamples with each subsample weighing approximately 200 g. Details of sample collection for the Feed Contaminants Program and the *Salmonella* Assignment can be found online (available at www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/ComplianceEnforcement/UCM113409.pdf; www.fda.gov/AnimalVeterinary/Products/AnimalFoodFeeds/Contaminants/ucm230837.htm).

Salmonella isolation and identification

From each of the 10 subsamples, approximately 37.5-g samples were aseptically weighed to form a 375-g composite sample for *Salmonella* analysis in FDA's microbiological laboratories. The presence of *Salmonella* was determined by using the methods described in Chapter 5 of the Bacteriological Analytical Manual (BAM, 2011). Each *Salmonella* isolate from the sample was further characterized by serotyping and antimicrobial susceptibility testing.

Serotyping

Serotyping was performed according to the procedure described in Identification and Serotyping of *Salmonella* (Brenner *et al*., 1998) in FDA's microbiological laboratories.

Antimicrobial susceptibility test

The antimicrobial susceptibility of the *Salmonella* isolates was tested in FDA's microbiological laboratories by using the Sensititre system (TREK Diagnostic Systems, Cleveland, OH), which is a microversion of the classic broth dilution method. For each isolate, MICs to 15 antimicrobial agents were determined and interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2010). The 15 antimicrobial agents tested were amoxicillin/clavulanic acid, ampicillin, cefoxitin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, norfloxacin sulfamethoxazole, sulfisoxazole, streptomycin, tetracycline, trimethoprim, and trimethoprim/sulfamethoxazole.

Statistical analysis

Statistical tests of significance were performed using SAS (SAS Institute, 2000). The chi-square test was performed to test the significant differences in the prevalence of *Salmonella* between the two time periods 2002–2006 and 2007–2009 by categories of animal versus plant-derived ingredients, and pet foods versus pet treats. Any test in which the p value was equal to or less than 0.05 was considered a significant difference.

Results

Prevalence of Salmonella

A total of 2,058 samples under the Feed Contaminants Program (2002–2009) and the *Salmonella* Assignment (2007–2009) were collected from complete animal feeds, feed

Table 1. Prevalence of *Salmonella* in the Different Categories of Animal Feeds Collected Under the Feed Contaminants Program in 2002–2009 and *Salmonella* Assignment in 2007–2009

Year	*Tested samples* (n)	*Positive samples* (n)	*Prevalence*[a] *(%)*
2002	187	34	18.2 (34/187)
2003	194	51	26.3 (51/194)
2004	150	22	14.7 (22/150)
2005	194	27	13.9 (27/194)
2006	144	23	16.0 (23/144)
Subtotal (2002–2006)	869	157	18.1[b] (157/869)
2007	284	28	9.8 (28/284)
2008	321	25	7.8 (25/321)
2009	584	47	8.0 (47/584)
Subtotal (2007–2009)	1,189	100	8.4[c] (100/1189)
Total	2,058	257	12.5 (257/2058)

[a]The sum of the prevalence is not equal to 100% due to rounding.
[b,c]*Salmonella* prevalence in 2007–2009 is significantly ($p \le 0.05$) lower than that in 2002–2006.

Table 2. *Salmonella* Prevalence in the Different Categories of Animal Feeds Collected Under the Feed Contaminants Program in 2002–2009 and *Salmonella* Assignment in 2007–2009

	Prevalence (%)	
Category	*2002–2006*	*2007–2009*
Complete feeds[c]	9.4[a] (34/363)[d]	5.6[a] (10/180)
Feed ingredients	30.9[a] (104/337)	19.4[b] (40/206)
Supplements for pets	18.8[a] (3/16)	7.1[a] (6/84)
Pet foods/treats	12.4[a] (19/153)	6.1[b] (44/719)
Total	18.1[a] (157/869)	8.4[b] (100/1189)

[a,b]Columns within the same raw with unlike superscripts differ significantly at $p \leq 0.05$.
[c]Complete feeds are poultry feeds, cattle feeds, swine feeds, horse feeds, medicated feeds, and feeds for minor species (e.g., rabbit, bird, and reptile).
[d]Number of sample positive/number of sample tested.

ingredients, pet foods, pet treats, and supplements for pets. As shown in Table 1, 257 of the 2,058 samples were positive for *Salmonella,* giving an overall prevalence of 12.5%. The overall prevalence of *Salmonella* in 2007–2009 (8.4%) is significantly ($p \leq 0.05$) lower than that in 2002–2006 (18.1%).

Prevalence of Salmonella *in different categories of animal feeds*

To identify the factors that contributed to the reduction of *Salmonella* prevalence from 2002 to 2009, animal feeds are separated into the following categories as shown in Table 2: complete feeds [poultry feeds, cattle feeds, swine feeds, horse feeds, medicated feeds, feeds for minor species (e.g. rabbit, bird and reptile)], feed ingredients, supplements for pets, and pet foods/treats. The results indicate that the prevalence of *Salmonella* of complete feeds, and supplements for pets, in 2007–2009, is not significantly lower than that in 2002–2006. However, the prevalence of *Salmonella* in feed ingredients and pet foods/treats in 2007–2009 was significantly ($p \leq 0.05$) lower than that in 2002–2006.

Prevalence of Salmonella *in categories of feed ingredients and pet foods/treats*

To further identify the factors that contributed to the reduction of *Salmonella* prevalence in categories of feed ingredients and pet foods/treats, the feed ingredients category was separated into animal-derived and plant-derived ingredients, and the pet foods/treats category was divided into pet foods (e.g., dog and cat food, aquarium fish food, raw meat, and raw poultry formulations for pets), and pet treats (e.g., rawhide bones, pig ears, dog biscuits). As shown in Figure 1A, the prevalence of *Salmonella* in animal-derived ingredients was significantly ($p \leq 0.05$) reduced from 66.1% in 2002–2006 to 41.3% in 2007–2009. The prevalence of *Salmonella* in animal-derived ingredients was also significantly ($p \leq 0.05$) higher than that in plant-derived ingredients in both time periods.

Figure 1B shows the differences of *Salmonella* prevalence in pet foods and pet treats. The prevalence of *Salmonella* in pet treats significantly ($p \leq 0.05$) declined from 12.3% in

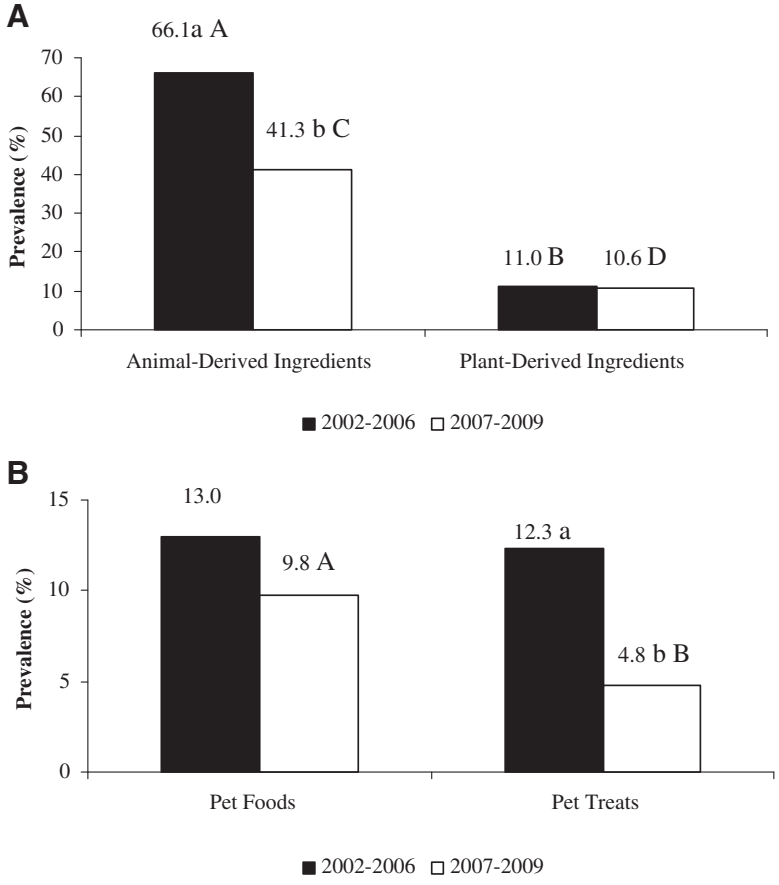

FIG. 1. Prevalence of *Salmonella* in feed ingredients and pet foods/treats collected under the Feed Contaminants Program in 2002–2009 and *Salmonella* Assignment in 2007–2009. **(A)** Prevalence of *Salmonella* in animal-derived ingredients and plant-derived ingredients. Significant ($p \leq 0.05$) difference of *Salmonella* prevalence in animal-derived ingredients between 2002–2006 and 2007–2009 is marked as a and b. Significant ($p \leq 0.05$) differences of *Salmonella* prevalence between animal-derived ingredients and plant-derived ingredients in 2002–2006 is marked as A and B. Significant ($p \leq 0.05$) differences of *Salmonella* prevalence between animal- and plant-derived ingredients in 2007–2009 is marked as C and D. **(B)** Prevalence of *Salmonella* in pet foods and pet treats. Significant ($p \leq 0.05$) difference of *Salmonella* prevalence in pet treats between 2002–2006 and 2007–2009 is marked as a and b. Significant ($p \leq 0.05$) difference in the *Salmonella* prevalence between pet foods and pet treats in 2007–2009 is marked as A and B.

2002–2006 to 4.8% in 2007–2009. Also, the prevalence of *Salmonella* in pet foods declined from 13.0% in 2002–2006 to 9.8% in 2007–2009, but this reduction was not statistically significant. It can also be noted that pet foods had a higher *Salmonella* prevalence than pet treats in both time periods; however, the difference was only significant ($p \leq 0.05$) in 2007–2009 (9.8% vs. 4.8%). Based on this observation, the significant decline in *Salmonella* prevalence in the categories of feed ingredients and pet foods/treats was due to the significant ($p \leq 0.05$) *Salmonella* reduction in animal-derived ingredients and pet treats.

Serotype

A total of 45 *Salmonella* serotypes were identified among the 257 *Salmonella* isolates obtained from this surveillance. The top 25 *Salmonella* serotypes are shown in Table 3 and are compared to the top 20 *Salmonella* serotypes identified from human infections in 2009 as reported by the CDC. *Salmonella* Senftenberg and *Salmonella* Montevideo were the most common serotypes (8.9%) among those identified. *Salmonella* Typhimurium and *Salmonella* Enteritidis were the most common serotypes identified in human infections. All the *Salmonella* Enteritidis and Typhimurium isolates in this surveillance were isolated from pet treats, such as pig ears. The data indicate that the most common serotypes identified from the animal feeds, feed ingredients, and pet foods/treats in this surveillance were not completely consistent with the most common serotypes causing human infections.

Table 3. Comparison of the 25 Most Common *Salmonella* Serotypes Found in the Different Categories of Animal Feeds Collected Under the Feed Contaminants Program in 2002–2009 and *Salmonella* Assignment in 2007–2009 to the 20 Most Common *Salmonella* Serotypes Found in Human Infections in 2009 Reported by the Centers for Disease Control and Prevention

	Animal feeds (2002–2009)		*Human (2009)*[a]	
Rank	*Serotype*	*%*	*Serotype*	*(%)*
1	Senftenberg	8.9	Enteritids	17.5
2	Montevideo*,[b]	8.9	Typhimurium	15.0
3	Mbandaka	8.6	Newport	9.3
4	Tennessee	6.2	Javiana	4.9
5	Typhimurium*	5.4	Heidelberg	3.5
6	I 4, [5], 1 2:i:-*	5.0	Montevideo	3.1
7	Schwarzengrund*	4.7	I 4, [5], 1 2:i:-	2.4
8	Anatum	4.3	Oranienburg	2.2
9	Agona*	3.5	Saintpaul	2.1
10	Johannesburg	3.5	Muenchen	2.0
11	Enteriditis*	3.1	Braenderup	1.8
12	Havana	3.1	Infantis	1.6
13	Cerro	2.7	Thompson	1.2
14	Oranienburg*	2.7	Mississippi	1.1
15	Arkansas	1.6	Paratyphi B var	1.1
16	Bredeney	1.6	Typhi	1.1
17	Cubana	1.6	Agona	1.0
18	Derby	1.6	Schwarzengrund	0.9
19	Alachua	1.2	Bareilly	0.7
20	Hadar*	0.8	Hadar	0.7
21	Weltevreden	0.8	Subtotal	72.8
22	Amager	0.8	All other serotypes	27.2
23	Muenchen*	0.8	Total	100
24	Kentucky	0.8		
25	Lille	0.4		
	Subtotal	82.5		
	All other serotypes	17.5		
	Total	100		

[a] *Salmonella* 2009 Annual Summaries, Table 1, Top 20 Reported Serotypes from Human by CDC (available at www.cdc.gov/ncezid/dfwed/PDFs/SalmonellaAnnualSummaryTables2009.pdf).

[b] *Salmonella* serotypes with * in the animal feed column are the ones also present in the human column.

Antimicrobial resistance

A total of 54 of the 257 (21.0%) *Salmonella* isolates were resistant to at least one antimicrobial. Of the 54 antimicrobial resistant *Salmonella* isolates, eight isolates were resistant to four or more antimicrobials. Resistance to tetracycline, sulfisoxazole, ampicillin, and nalidixic acid was most common, whereas resistance to ciprofloxacin, trimethoprim, and norfloxacin was least common, as shown in Figure 2A. In addition, *Salmonella* Enteriditis and *Salmonella* Typhimurium isolated from pet treats were among the most antimicrobial resistant isolates with resistance rates of 88% and 71%, respectively, as shown in Figure 2B, althought the number of *Salmonella* Enteritidis and Typhimurium examined were relative small.

Discussion

Most significantly is the finding that the overall *Salmonella* prevalence in animal feeds (complete animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets) was reduced from 18.2% in 2002 to 8.0% in 2009. A similar survey conducted in Great Britian in 1993–2006 indicates that the *Salmonella* contamination rate in animal feedstuffs and ingredients decreased from 3.8% in 1993 to 1.1% in 2006 (Papadopoulou *et al.*, 2009). Other findings suggest that *Salmonella* contamination of complete animal feed (finished feed) is common, with studies in the United States and in European countries reporting that *Salmonella* contamination rates in complete animal feed (finished feed) range from 1.1% to 41.7% (Veldman *et al.*, 1995; Davies *et al.*, 1997; Whyte *et al.*, 2003). Our results indicate that the *Salmonella* reduction is mainly a result of the reduction of *Salmonella* prevalence in feed ingredients, especially animal-derived ingredients, and pet foods/treats, especially pet treats. In comparison to the FDA CVM 1994 survey in which 82% of animal-derived ingredients and 37% of plant-derived ingredients were positive for *Salmonella* (McChesney, 1995), the results indicate that the prevalence of *Salmonella* in animal-derived ingredients (66.1% in 2002–2006 and 41.3% in 2007–2009) and plant-derived ingredients (11.1% in 2002–2006 and 10.6% in 2007–2009) have been further reduced. The significant reduction of *Salmonella* prevalence in feed ingredients was a noteworthy finding because this reduction is likely to have a large impact on the contamination of finished feeds, pet foods, and pet treats. This finding reflects the FDA CVM survey in 1994 that found when the feed ingredients used for making a complete feeds were positive for *Salmonella,* the complete feeds were also positive 30% of the time (McChesney, 1995). Furthermore, the reason that a greater reduction was observed in animal-derived

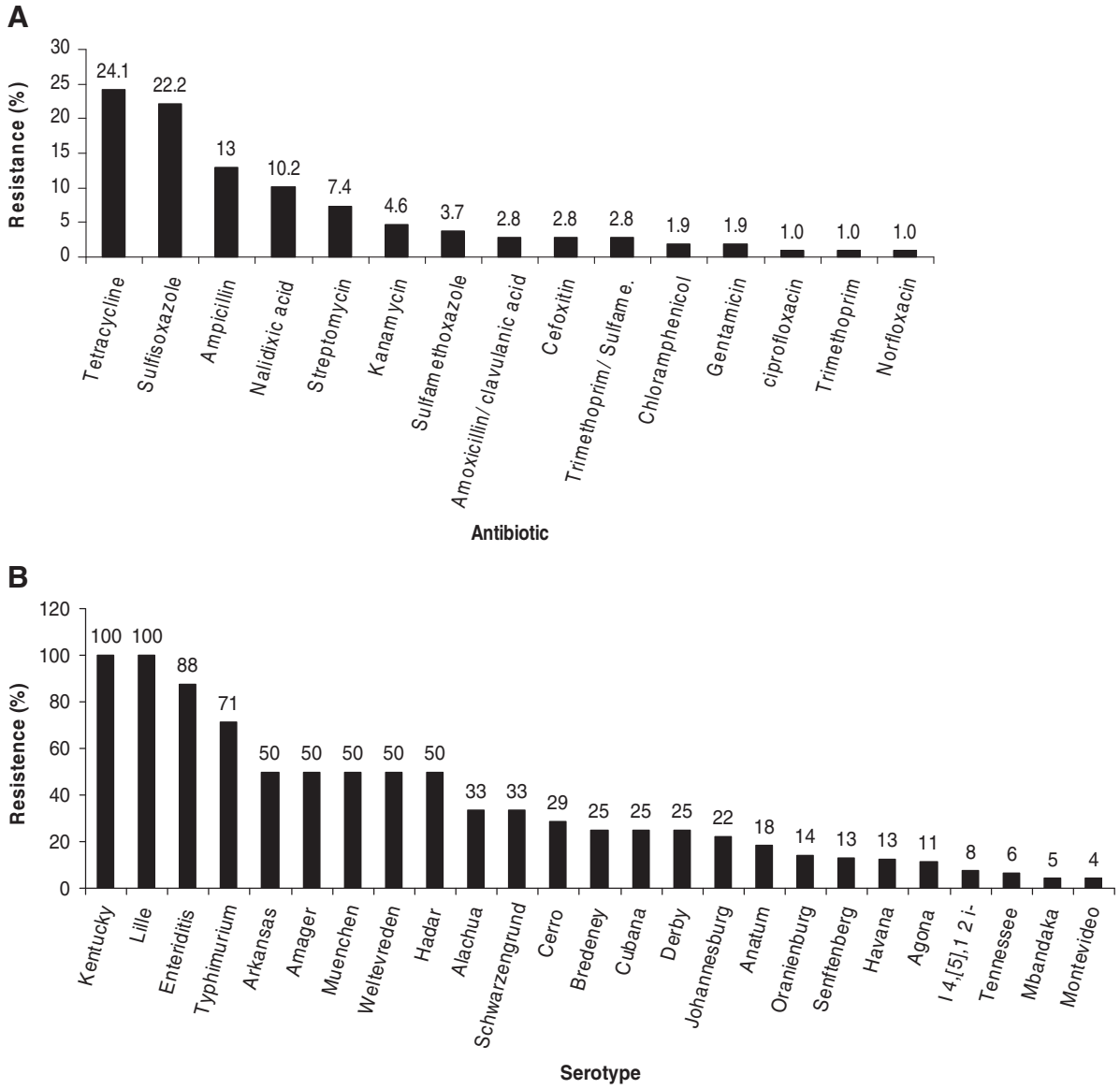

FIG. 2. Comparison of antimicrobial susceptibility of the 25 most common *Salmonella* serotypes found in the different categories of animal feeds collected under the Feed Contaminants Program in 2002–2009 and Salmonella Assignment in 2007–2009. **(A)** Antibiotic resistance profiles by antibiotic for the *Salmonella* isolates found in the different categories of animal feeds collected under the Feed Contaminants Program in 2002–2009 and Salmonella Assignment in 2007–2009. **(B)** Antibiotic resistance profiles by serotype for the Salmonella isolates found in the different categories of animal feeds collected under the Feed Contaminants Program in 2002–2009 and Salmonella Assignment in 2007–2009.

ingredients than in plant-derived ingredients could be that animal-derived ingredients are more carefully controlled and scrutinized because not only are they at risk for bacterial contamination by organisms such as *Salmonella*, but also because they are at risk for hazards such as bovine spongiform encephalopathy (Hamilton, 2002). The significant reduction of *Salmonella* in pet treats could be due to a greater awareness of the issue of *Salmonella* in Direct-Human-Contact Feeds, including pet foods and pet treats.

Even though there was a significant reduction of *Salmonella* prevalence in animal-derived ingredients, it was still significantly higher than that in plant-derived ingredients. The result is consistent with that in the 1994 FDA CVM survey (McChesney, 1995) that found animal-derived ingredients consistently had a higher prevalence of *Salmonella* than plant-derived ingredients 82% and 37%, respectively. Pet foods can contain up to 60% (w/w) animal-derived ingredients in comparison to complete animal feeds (finished animal feeds) which contain only approximately 2% (w/w) animal-derived ingredients (Brookes, 2001; Hendriks *et al*., 1999). These results may explain why pet foods and pet treats had a higher *Salmonella* prevalence as compared to complete animal feed. This observation raises public health concerns because pet foods and pet treats are Direct-Human-Contact Feeds from which *Salmonella* can be spread directly to people. Therefore, the public needs to be aware of this risk and take necessary precautions such as thoroughly washing their hands after handling pet foods and pet treats. Additional precautions

include health care professionals and veterinarians advising pet owners about the health risks of handling pet foods and pet treats, and prevention methods. The results also stress the need for pet foods and pet treats manufacturers to implement validated bacterial kill steps (Health Canada, 2006).

The most common serotypes identified from the animal feed in this surveillance were not consistent with the most common serotypes causing human infections. This is logical because humans can be exposed to *Salmonella* from a great varity of sources. In addition, part of the difference in the panorama of serotypes may be because every serotype differs in its ability to cause human illness (Sarwari *et al.,* 2001) and to resist eradication methods such as heat, irradiation, and desiccation (Pires *et al.,* 2010). For example, *Salmonella* Senftenberg is frequently isolated from feeds and feed ingredients, as indicated in our results, because it is more resistant to these eradication methods (Lofstrom *et al.,* 2006; Papadopoulou *et al.,* 2009). However, six of the top 10 serotypes found in humans were also isolated from the feed showing that *Salmonella* in animal feed may be one of the potential sources of salmonellosis in humans.

Finally, the study found that *Salmonella* Enteritidis and *Salmonella* Typhimurium isolated from pet treats were highly resistant to antimicrobials with resistance rates 88% and 71%, respectively, althought the number of *Salmonella* Enteritidis and Typhimurium examined were relatively small.

This is a significant public health concern because *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most common serotypes found in human infections, accounting for the most human illnesses in 2009 (17.5% and 15.0%, respectively). Therefore, treating infections associated with these serotypes could become more difficult, and they could pose an even higher public health burden.

Conclusion

The results presented here suggest that progress in the reduction of *Salmonella* in animal feeds, feed ingredients, pet foods, pet treats, and supplements for pets is being made. Our findings provide the animal feed industries with *Salmonella* prevalence information that can be used to address *Salmonella* contamination problems. Our findings can also be used to educate pet owners on the importance of safely handling pet foods and treats to prevent salmonellosis.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Xin Li, Ph.D.
Division of Animal Feeds, HFV-222
Office of Surveillance and Compliance
Center for Veterinary Medicine
U.S. Food and Drug Administration
7519 Standish Place
Rockville, MD 20855

E-mail: xin.li@fda.hhs.gov