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Dissemination of *Salmonella enterica* subsp. *enterica* Serovar Typhimurium var. Copenhagen Clonal Types through a Contract Heifer-Raising Operation

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***Salmonella enterica* subsp. *enterica* serovar Typhimurium var. Copenhagen isolates from a heifer-raising operation and from 11 dairy herds that had their calves contracted to the heifer-raising operation were examined for their phenotypic and genotypic characteristics. Results of the study showed that the heifer-raising operation could serve as a clearinghouse for *Salmonella* serovar Typhimurium var. Copenhagen and perhaps other *Salmonella* serotypes.**

Salmonella enterica subsp. *enterica* serovar Typhimurium var. Copenhagen is an O:5-negative variant of *Salmonella* serovar Typhimurium which was primarily reported to be found in pigeons. It is now frequently isolated from cattle, swine, and other animals (7). The U.S. Department of Agriculture's National Animal Health Monitoring System for Enteric Bacteria reported that over a 7-year period (1997 to 2003), *Salmonella* serovar Typhimurium, which includes variant Copenhagen, was the most predominant serotype and accounted for 16.9% of the total number of isolates (n = 40,120) examined. Over this period, 6,695 isolates were serotyped as *Salmonella* serovar Typhimurium, and of these isolates, 51% were determined to be *Salmonella* serovar Typhimurium var. Copenhagen (15).

In June of 1998, a heifer-raising operation in Pennsylvania with recurrent problems associated with calf mortality sought the assistance of the Field Investigation Group at Pennsylvania State University to address the issue. At the beginning of August of 1998, the veterinarians attending the heifer-raising operation and 18 dairy herds that received heifers from the heifer-raising operation were asked to submit samples (fecal and tissue samples) for bacteriological analysis from all clinical cases suggestive of salmonellosis. Between September 1998 and October 2000, samples from 324 calves, heifers, and lactating cattle from the heifer-raising operation and 11 dairy herds were cultured for *Salmonella* using the protocol followed by Pennsylvania Animal Diagnostic Laboratory for isolation and identification of *Salmonella*. *Salmonella* isolates were serotyped at the National Veterinary Services Laboratory, Ames, Iowa.

Salmonella serovar Typhimurium var. Copenhagen isolates (n = 42) were screened for antibiotic resistance using a disk diffusion assay, and antibiotic resistance or susceptibility was determined using the interpretive criteria defined by NCCLS (16). Genes for beta-lactam, tetracycline, and florfenicol resistance and for class 1 integron were identified using techniques described previously (3, 4, 11, 17, 20, 23, 24). *Salmonella* serovar Typhimurium var. Copenhagen isolates were subtyped using the 1-day pulsed-field gel electrophoresis (PFGE) protocol reported by Gautom (8). Epi-info 2002 (Centers for Disease Control and Prevention, Atlanta, GA), a database and statistics system for epidemiology on microcomputers, was used for performing χ^2 tests and odds ratio analysis.

A total of 62 *Salmonella* isolates belonging to six serotypes, including *Salmonella* serovar Typhimurium, *Salmonella* serovar Typhimurium var. Copenhagen, *Salmonella enterica* serovar Muenchen, *Salmonella enterica* serovar Newport, *Salmonella enterica* serovar Heidelberg, and *Salmonella enterica* serovar Montevideo, were isolated in this study (Table 1). *Salmonella* serovar Typhimurium var. Copenhagen accounted for 42 of the 62 (68%) *Salmonella* isolates. These isolates have been previously isolated from calves, heifers, and lactating cows in Pennsylvania (6, 18).

On the dairy farm, the likelihood of isolating *Salmonella* from a sick heifer was 2.6-fold higher than that for isolation from sick calves. With regard to *Salmonella* serovar Typhimurium var. Copenhagen, the likelihood of isolating *Salmonella* serovar Typhimurium var. Copenhagen from calves on the heifer-raising operation was 5.3-fold higher than that for isolation from heifers, while on the dairy farm, *Salmonella* serovar Typhimurium var. Copenhagen was more likely (2.3-fold higher likelihood) to be isolated from heifers than from calves (Table 1). Transition of animals from one environment to another (e.g., from dairy farm to heifer-raising operation and vice versa), change in nutrition (protein and energy content), and interaction with other animals (access to stall, water, and feed troughs) in the cohort could result in a cascade of events that could induce stress, making the animal more susceptible to infectious diseases (5, 9, 10, 12, 13). These sets of complex interactions could perhaps explain the higher *Salmonella* infection rates of calves that were transferred to the heifer-raising operation and of heifers that returned to their dairy herds.

The 42 isolates of *Salmonella* serovar Typhimurium var.

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TABLE 1. *Salmonella* serotypes isolated from calves and cows

Source of isolates	Cattle group (no. of animals tested)	No. of isolates (no. of farms)						Total no. of isolates
		Serovar Typhimurium	Serovar Typhimurium var. Copenhagen	Serovar Muenchen	Serovar Newport	Serovar Heidelberg	Serovar Montevideo	
Heifer-raising operation[a]	Calves (71)	2	8	0	1	2	0	13
	Heifers (86)	0	2	1	0	1	2	6
	Total	2	10	1	1	3	2	19
Dairy farms[b]	Calves (91)	1	12	0	1	1	1	16
	Heifers (76)	2	20	1	0	0	4	27
	Total	3 (2)	32 (11)	1 (1)	1 (1)	1 (1)	5 (3)	43
Total isolates		5	42	2	2	4	7	62

[a] For all isolates from animals in the heifer-raising operation, the χ^2 value was 4.67, the *P* value was 0.0307 (a *P* value less than 0.05 is statistically significant), and the odds ratio was 2.99 (confidence interval, 0.98 to 9.45). For the serovar Typhimurium var. Copenhagen isolates from animals in the heifer-raising operation, the χ^2 value was 5.18, the *P* value was 0.0228, and the odds ratio was 5.33 (confidence interval, 1.00 to 37.76).

[b] For all isolates from animals on dairy farms, the χ^2 value was 6.93, the *P* value was 0.0084 (a *P* value less than 0.05 is statistically significant), and the odds ratio was 2.58 (confidence interval, 1.19 to 5.63). For the serovar Typhimurium var. Copenhagen isolates from animals on dairy farms, the χ^2 value was 4.61, the *P* value was 0.0318, and the odds ratio was 2.35 (confidence interval, 1.00 to 5.61).

Copenhagen belonged to seven PFGE profiles. Of the 7 PFGE profiles, types STC1 and STC2 accounted for 12 (28.5%) and 21 (50%) of the isolates, respectively (Table 2). Twenty-eight of 42 *Salmonella* serovar Typhimurium var. Copenhagen isolates showed the presence of the bla_{TEM} gene and 12 isolates showed the presence of bla_{PSE}, while 2 isolates showed the presence of the bla_{CMY} gene; *tetA*, *tetG*, and *tetB* were detected in 26, 12, and 4 isolates, respectively. Twenty-five isolates, including 15 isolates with *floST* and 10 isolates with the *floR* gene, harbored genes for florfenicol resistance (Table 2). Integron 1 was present in 36 of 42 *Salmonella* serovar Typhimurium var. Copenhagen isolates. DNA sequence analysis showed that PCR-amplified DNA fragments of ~1,000, 1,100, and 1,300 bp were genes coding for spectinomycin resistance (*aadA1*), beta-lactamase (bla_{PSE}), and trimethoprim resistance (*dhfrA*), respectively (Table 2).

Analysis of beta-lactamase, tetracycline-resistant, florfenicol-resistant, and integron 1 genes resulted in the identification of 14 resistance genotypes (Table 2). The PCR-generated antibiotic resistance genes *floR* and *floST*, which confer resistance to florfenicol and chloramphenicol, have previously been identified in *S. enterica* serovar Typhimurium DT104 and *Escherichia coli* (3, 22). In our study, isolates that had *tetG* also had the *floR* gene. A similar observation was made by Baucheron et al. (1). The characteristic ACSSuT and ACSuT resistance profiles were observed in 38 and 19% of the isolates, respectively (Table 2). The ACSSuT and ACSuT resistance profiles have been used as diagnostic markers for monitoring multidrug resistance of *Salmonella* serovar Typhimurium DT104 from animal and human sources (2, 14, 23).

Based on phenotypic and genotypic characteristics, the 42 isolates were classified into 19 clonal types (Tables 2 and 3). Six

TABLE 2. Phenotypic and genotypic characteristics of *Salmonella* serovar Typhimurium var. Copenhagen isolated from dairy cattle with salmonellosis

Resistance genotype	PFGE profile	Antibiogram profile	Clonal type	No. of isolates in:		Total no. of isolates
				Dairy herd (no. of herds in which isolates were found)	Heifer-raising operation	
bla_{PSE} *tetG floR aadA1*	STC1	ACTSpF	1	1 (1)		1
bla_{PSE} *tetG floR aadA1 dhfrA*	STC1	ACSSuTSpF	2	1 (1)	1	2
bla_{PSE} *tetG floR aadA1*	STC1	ACSuTSpF	3	1 (1)	1	3
bla_{PSE} *tetG aadA1*	STC1	ATSp	4	4 (3)	1	5
bla_{PSE} *tetG floST aadA1 dhfrA*	STC1	ACSSuTSpF	5	1 (1)		1
bla_{TEM} *tetA floR aadA1 dhfrA*	STC2	ACSSuTSpF	6	1 (1)		1
bla_{TEM} *tetA aadA1*	STC2	ATSp	7	1 (1)		1
bla_{TEM} *tetA aadA1*	STC2	ASTSp	8	3 (3)	2	5
bla_{TEM} *tetA aadA1*	STC2	ASSuTSp	9	2 (1)		2
bla_{TEM} *tetA floST aadA1*	STC2	ACSTSpF	10	1 (1)	2	3
bla_{TEM} *tetA floST aadA1 dhfrA*	STC2	ACSSuTSpF	11	5 (4)	3	8
bla_{TEM} *tetA floST aadA1*	STC2	ACSuTSpF	12	1 (1)		1
bla_{TEM} *tetA floST*	STC3	ACSSuTF	13	1 (1)		1
bla_{TEM} *tetA aadA1*	STC4	ASSuTSp	14	1 (1)		1
bla_{TEM} *tetA floR*	STC5	ACSSuTF	15	1 (1)		1
bla_{TCMY} *tetA floR aadA1 dhfrA*	STC6	ACSSuTSpFCe	16	1 (1)		2
bla_{TEM} *tetB*	STC7	ASSuT	17	1 (1)		1
bla_{TEM} *tetB*	STC7	ASuT	18	1 (1)		2
bla_{TEM} *tetB floST*	STC7	ACSSuTF	19	1 (1)		1

TABLE 3. Distribution of *Salmonella* serovar Typhimurium var. Copenhagen clonal types

Clonal type	Heifer-raising operation		Characteristic of heifer reception		
	Date (mo/yr)	Animal (age in days)	County (no. of farms)	Date (mo/yr)	Animal (age)[a]
1			A (2)	Aug., '99	Calf (35)
2	Sept., '98	Calf (15)	B (1)	June, '99	Heifer (c-5)
3	Oct., '98	Calf (21)	C (1)	Oct., '99	Heifer (1st lac., 3 DIM)
4	Nov., '98	Heifer (695)	D (1)	Sept., '99	Calf (28)
			D (2)	Sept., '99	Cow (2nd lac.)
			D (2)	Jan., '00	Heifer (1st lac., 10 DIM)
			E (1)	Sept., '99	Heifer (702)
			E (1)	Oct., '99	Heifer (120)
5			F (1)	Jan., '99	Heifer (1st lac., 2 DIM)
6			A (2)	Nov., '98	Calf (21)
7			D (1)	Feb., '99	Cow (3rd lac.)
8	Dec., '98	Calf (21)	G (1)	July, '99	Heifer (c-4)
	Jan., '99	Calf (35)	H (1)	July, '99	Cow (2nd lac.)
			H (1)	Aug., '99	Calf (28)
9			B (1)	June, '99	Cow (3rd lac.)
			B (1)	Sept., '99	Calf (28)
10	Nov., '98	Calf (7)	1 (1)	Sept., '99	Heifer (c-0)
	Dec., '98	Calf (14)			
11	Dec., '98	Calf (14)	F (1)	Sept. '99	Heifer (c-1)
	Feb., '99	Heifer (684)	D (1)	July, '99	Calf (14)
	July, '99	Calf (14)	D (2)	Dec., '99	Heifer (1st lac., 14 DIM)
			D (2)	Feb., '00	Cow (3rd lac.)
			G (1)	Oct., '98	Heifer (1st lac., c-2)
12			J (1)	Oct., '98	Calf (23)
13			J (1)	Oct., '98	Cow (1st lac.)
14			F (1)	Mar., '99	Cow (2nd lac.)
15			C (1)	Oct., '99	Calf (21)
16			C (1)	Sept., '99	Calf (42)
			C (1)	Oct., '99	Cow (1st lac.)
17			D (1)	Sept., '99	Cow (3rd lac.)
18			A (2)	Aug., '99	Heifer (c-10)
			A (2)	Aug., '99	Cow (2nd lac.)
19			D (2)	Sept., '98	Calf (42)

[a] Ages are given in days, in days before calving (e.g., c-5 is 5 days before calving), in days in milk (DIM), and/or by lactation (lac.).

of the 19 clonal types from animals on the heifer-raising operation were also observed in 9 of 11 dairy herds. It was observed that most of the isolates from the heifer-raising operation were from calves with salmonellosis, while the same clonal types on dairy herds were isolated mostly from heifers ($n = 10$) rather than from calves ($n = 3$) and lactating cows ($n = 3$). Clonal types that were detected in the heifer-raising operation were observed 6 to 12 months later in the dairy herds. Thirteen of the 19 clonal types were detected exclusively in dairy herds; these isolates were mostly from calves ($n = 7$) or lactating cattle (n = 7) rather than from heifers ($n = 2$) (Table 3). Recently, Hume et al. (13) observed multiple serotypes and genotypes in a herd, which suggested multiple sources of *Salmonella* contamination. The findings of their study revealed that dairy cows could serve as asymptomatic carriers of *Salmonella*.

Contract heifer raising requires meticulous planning and implementation of rigorous biosecurity practices. Biosecurity deals with management practices that protect the herd from the entry of new diseases and minimize the spread and/or adverse effects of diseases in the herd (21). A contract heifer-raising operation acquires calves from several farms that are commingled. This is the single most important risk factor for the introduction of new diseases on the premises. More importantly, the organisms may leave the premises, with healthy heifers serving as vehicles. Biosecurity is one of the major issues facing professional heifer growers who have multiple clients. Most contract raising operations include biosecurity practices to address brucellosis, persistent bovine viral diarrhea disease, and Johne's disease (19). Based on the findings of our study, it is felt that biosecurity practices focused on the prevention and control of enteric pathogens yet remain to be addressed adequately.

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