

Antimicrobial resistance of fecal *Salmonella* spp. isolated from all phases of pig production in 20 herds in Alberta and Saskatchewan

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Abstract

Salmonella spp. ($n = 468$), isolated from the feces of sows, nursery, and grow-finish pigs in 20 farrow-to-finish herds in Alberta and Saskatchewan, were tested for susceptibility to 16 antimicrobials. No resistance was identified to amikacin, amoxicillin-clavulanic acid, ceftiofur, ceftriaxone, ciprofloxacin or nalidixic acid, and less than 1% of the isolates were resistant to cefoxitin and gentamicin. Isolates were most commonly resistant to tetracycline (35%) and sulfamethoxazole (27%). Overall, 59% of the *Salmonella* were susceptible to all 16 drugs (pansusceptible). Isolates from sows were more likely to be pansusceptible than isolates from nursery or grow-finish pigs. Resistance to 2 or more drugs occurred in 29% of the isolates and was significantly more likely to occur in *Salmonella* from nursery pigs than from sows. The odds of resistance to 4 of the drugs, streptomycin, ampicillin, kanamycin and cephalothin, were significantly higher in isolates from nursery pigs than grow-finish pigs, while the odds of resistance to 2 drugs, tetracycline and streptomycin, were higher in *Salmonella* from nursery pigs than from sows. More age-specific risk factor studies are needed to investigate these differences between production phases.

Résumé

Des isolats de *Salmonella* spp. ($n = 468$), provenant des fèces de truies, de porcelets en pouponnière et de porcs en croissance-finition prélevés dans 20 troupeaux de naisseur-finiisseur en Alberta et Saskatchewan, ont été testés pour leur sensibilité à 16 antimicrobiens. Aucune résistance ne fut détectée envers les antibiotiques suivants : amikacine, amoxicilline-acide clavulanique, ceftiofur, ceftriaxone, ciprofloxacin et acide nalidixique; et moins de 1 % des isolats étaient résistants au cefoxitin et à la gentamicine. Les isolats étaient résistants le plus fréquemment à la tétracycline (35 %) et au sulfaméthoxazole (27 %). Globalement, 59 % des *Salmonella* étaient sensibles aux 16 antimicrobiens (pansensible). Les isolats provenant des truies étaient plus susceptibles d'être pansensibles que les isolats des animaux en pouponnière ou en croissance-finition. Une résistance à 2 antimicrobiens ou plus a été notée chez 29 % des isolats et étaient significativement plus probable de se produire chez les animaux en pouponnière que chez les truies. Les probabilités de résistance à 4 antimicrobiens (streptomycine, ampicilline, kanamycine et céphalothine) étaient significativement plus élevées chez les isolats provenant des porcs en pouponnière que les animaux en croissance-finition, alors que les probabilités de résistance à 2 antimicrobiens (tétracycline et streptomycine) étaient plus élevées pour les *Salmonella* provenant des animaux en pouponnière que chez les truies. Des études supplémentaires sur les facteurs de risque reliés à l'âge sont nécessaires pour comprendre ces différences entre les phases de production.

(Traduit par Docteur Serge Messier)

Introduction

Salmonella spp. are the 2nd leading cause of bacterial foodborne disease in Canada. Approximately 6000 to 9000 cases of human salmonellosis are reported annually, and for each reported illness 13 to 37 cases remain unreported (1–3). Antimicrobial resistant *Salmonella* can cause even greater morbidity than their susceptible counterparts due to treatment failure, increased infection severity, and increased rates of disease in people taking antimicrobials for other reasons (4–7). Although pork is not a major cause of salmonellosis in North America, it has been responsible for disease outbreaks of multi-resistant *Salmonella* in humans elsewhere (8–10).

Most *Salmonella* infections are acquired from contaminated food; therefore, studying antimicrobial resistance (AMR) in live, close-to-market pigs indirectly estimates the potential for cross-contamination at slaughter and the risk to consumers from resistant organisms in pork (11,12). In Canada, *Salmonella* AMR data are available from pigs at slaughter (13–15); however, these data may differ from those acquired on-farm because AMR patterns and serovars can change after transport and lairage (16). Extending AMR investigations to other pig categories, such as sows and nursery pigs, might improve our understanding of the occurrence and spread of AMR within pig production systems. This in turn, could lead to the identification of possible control measures. In Canada, on-farm *Salmonella* AMR

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data from healthy pigs are currently limited to a longitudinal study of finishing pigs from Alberta (17). The main goal of this study was to investigate and describe the AMR profiles of *Salmonella* isolates from apparently healthy nursery pigs, grow-finish pigs, and sows in 20 herds in Alberta and Saskatchewan.

Materials and methods

Herd and sample selection

Herd selection has been described in detail (18,19). Briefly, a convenience sample of 20 farms participated in this study. Ten herds were enrolled with knowledge of the presumed *Salmonella* status to address other research objectives (20). Seven herds were enrolled as *Salmonella*-positive and 3 *Salmonella*-negative based on clinical disease or test results in the previous 12 mo. The principle investigator and laboratory personnel were blind to the presumed herd status. The remaining 10 herds, with an unknown *Salmonella* status, were selected from as many veterinarians as possible in order to minimize clustering by geography and management practices. Each herd was visited once between May and September 2004. Fresh fecal samples were collected from pens selected using a random numbers table. Pooled pen samples (PPS) consisted of feces from 5 pigs for a pooled sample weight of approximately 75 g. In the 10 herds of unknown *Salmonella* status, 25 PPS were collected from grow-finish pigs. In the 10 presumed-known *Salmonella* status herds, samples were collected from each phase of pig production: 20 PPS from sows, 30 PPS from nursery pigs, and 30 PPS from grow-finish pigs. In addition, 30 individual samples were collected from grow-finish pigs and 10 from sows.

Laboratory methods

Salmonella isolation

All samples were shipped on ice to 1 of 3 laboratories within 24 h of collection. Samples from 10 herds were cultured for *Salmonella* by Agri-Food Laboratories Branch (AFLB), Food Safety Division of Alberta Agriculture, Food and Rural Development, Edmonton, Alberta; samples from 4 herds by Prairie Diagnostic Services (PDS), University of Saskatchewan, Saskatoon, Saskatchewan; and samples from 6 herds by Laboratory Services Division, University of Guelph, Guelph, Ontario.

AFLB Food Safety Division of Alberta Agriculture, Edmonton, Alberta

Salmonella Typhimurium ATCC 14028 was used as the quality control organism. Unless otherwise specified, the incubation temperature was 35°C. A 10 g fecal aliquot per sample was pre-enriched in 90 mL of buffered peptone water (BPW) for 20 to 24 h. From the BPW, 0.1 mL was transferred into 10 mL Rappaport-Vassiliadis (RV) enrichment broth and incubated at 42°C for 24 h. Concurrently, 1.0 mL was transferred into 10 mL of tetrathionate (TT) enrichment broth containing 0.2 mL of iodine solution and incubated for 24 h. Aliquots from the RV and TT broths (0.15 mL each) were pooled and screened by real-time polymerase chain reaction (R-PCR) for the presence of

Salmonella (21). Following incubation, 10 µL of RV broth and 10 µL of TT broth were transferred to xylose-lysine-tergitol 4 Agar (XLT4) and Rambach agar plates and incubated for 24 to 48 h. The TT broth (0.1 mL) was transferred to 3 sites on modified semi-solid Rappaport-Vassiliadis (MSRV) (Difco Laboratories, Oakville, Ontario) media and incubated at 42°C for up to 72 h. Each MSRV plate with a halo was subcultured onto XLT4 and RAM plates. Samples positive by R-PCR, but culture negative, were tested using immunomagnetic separation (IMS) technology (Dynabeads anti-*Salmonella*; Dynal Biotech, ASA, Oslo, Norway). Beads were enriched in TT broth and processed as described previously. In general, 1 suspect *Salmonella* colony per sample was selected for further characterization unless morphologically different colonies were identified, in which case both were harvested. Isolates were initially screened with triple sugar iron agar (TSI), lysine iron agar (LIA), and urea agar slants, and purity checked using 1/4 MacConkey agar and blood agar plates, respectively. Isolates were further screened with *Salmonella* Poly 0/01 agglutination (Denka Seiken, Tokyo Japan) and atypical colonies were tested with Vitek GNI or API-20E (bioMérieux Canada, Montreal, Quebec). Presumptive *Salmonella* isolates were frozen at -70°C in defibrinated sheep's blood and sent to the Office International des Épizooties (OIE) Reference Laboratory for Salmonellosis, in Guelph, Ontario for confirmation by serotyping.

Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, Saskatchewan

The protocol developed by AFLB was utilized by PDS with minor modifications; R-PCR and IMS were not used. Screening differed in that suspect colonies were incubated for 2 to 4 h in trypticase soy (TS) broth and subsequently tested with a citrate slant rather than LIA. Isolates were sent in TS broth to the Saskatchewan Health Provincial Laboratory for serotyping.

Laboratory Services Division, University of Guelph, Guelph, Ontario

Health Canada's standard *Salmonella* isolation protocol (22) was used with the IMS technology (Dynabeads anti-*Salmonella*; Dynal Biotech, ASA). Suspect colonies were tested with TSI, LIA, and urea slants to confirm *Salmonella* status, frozen at -80°C in glycerol, and sent to the OIE Reference Laboratory for Salmonellosis, Guelph, Ontario for serological testing.

Serotyping and phagotyping

Office International des Épizooties (OIE) Reference Laboratory for Salmonellosis, Guelph, Ontario

The O antigens of the *Salmonella* isolates were determined by slide agglutination (23). The H antigens were identified using a microtechnique that employs microtitre plates (24). The antigenic formulae of Le Minor and Popoff (25) were used to name the serovars. The standard phagotyping technique described by Anderson and Williams (26) was followed. The phagotyping scheme and phages for *Salmonella* Typhimurium, developed by Callow (27), and further extended by Anderson (28), Anderson et al (29), and Ward (30), were obtained from the International Centre for Enteric Phage Typing (ICEPT), Central Public Health Laboratory, Colindale, United Kingdom via the National Microbiology Laboratory (NML), Public

Health Agency of Canada, Winnipeg, Manitoba. The *Salmonella* Heidelberg phagotyping scheme and phages were supplied by the NML (31). Isolates that reacted with the phages but did not conform to any recognized phage type were considered atypical. Strains that did not react with any of the typing phages were considered untypable.

Saskatchewan Health Provincial Laboratory, Regina, Saskatchewan

The O antigens of the *Salmonella* isolates were determined by slide agglutination and the H antigens were identified by a broth culture method (32). The antigenic formulae of Le Minor and Popoff were used to name the serovars (25). Samples identified as *Salmonella* Typhimurium (including var. Copenhagen), *Salmonella* Heidelberg or *Salmonella* Enteritidis were sent to the OIE Reference Laboratory for Salmonellosis, in Guelph, Ontario for phagotyping as described previously.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted by AFLB and PDS using a broth microdilution technique following Clinical and Laboratory Standards Institute (CLSI) guidelines (33,34). Antimicrobial susceptibility testing methods have been described in detail (18,19).

Data comparisons and statistical analysis

Culture, serovar, and minimal inhibitory concentration (MIC) data were maintained in a relational database (Microsoft Access; Microsoft Corporation, Redmond, Washington, USA). Intermediate MIC values were categorized as susceptible for all analyses (33,34). Isolates susceptible to all drugs in the panel were designated 'pansusceptible'. This definition does not imply isolates are susceptible to drugs not on the panel. Isolates resistant to 2 or more drugs were designated 'multiresistant' (14,17,35).

Descriptive analyses were conducted using commercially available software (Microsoft Excel; Microsoft Corporation, Redmond, Washington, USA). All statistical analyses accounted for clustering of resistance within herds through generalized estimating equations (GEE) (PROC GENMOD, SAS for Windows version 9.1; SAS Institute, Cary, North Carolina, USA). All models had a binary outcome, logit-link function, and an exchangeable correlation structure. Null binomial response models estimated the prevalence of pansusceptibility, multiresistance, and resistance to each drug. From each model the intercept (β_0) and 95% confidence intervals (CI) were used to calculate population-average prevalence estimates using the formula $[1 + \exp(-\beta_0)]^{-1}$ (36). Univariate logistic regression examined the source of isolates (production phase) as a predictor for the following outcomes: resistance to each drug, pansusceptibility, and multiresistance. Four additional sets of univariate analyses explored potential biases introduced by study design: knowledge of herd *Salmonella* status, herd size, individual sample versus PPS, and laboratory performing isolation. These factors, which were known prior to susceptibility testing, were each considered as predictors for drug resistances observed in more than 5% of the isolates. Associations between variables of interest and outcomes were reported as an odds ratio (OR = $\exp(\beta)$) with 95% CI. All associations with $P < 0.05$ were reported as significant (36).

Results

Salmonella was identified in 14 herds with a range of 2 to 95 positive samples per herd. Overall, 452 (32%) of the samples were positive for *Salmonella* and 16 samples had 2 distinct isolates harvested, resulting in 468 isolates for susceptibility testing. Thirty serovars were identified with 9 accounting for 89% of the isolates. These 9 serovars were each identified in at least 2 herds and, except for serovar Banana, were found in every production phase.

The highest prevalence of resistance was to tetracycline. No isolates were resistant to 6 drugs, including amikacin (Table I). However, the amikacin status of 1 isolate was indeterminable because the dilution range did not cross the breakpoint and the MIC was greater than the dilution range. Almost 85% of the isolates from 6 of the top 9 serovars were pansusceptible (Table II). In contrast, only 8% of *Salmonella* Mbandaka, 20% of *Salmonella* Typhimurium var. Copenhagen, and 55% of *Salmonella* Derby were pansusceptible (Table II).

Salmonella was isolated from 32% of the nursery samples, 28% of the grow-finish samples, and 47% of the samples from sows (Table III). A higher percent of nursery, compared to grow-finish or sow, isolates demonstrated resistance. This pattern was evident across all drugs except trimethoprim-sulfamethoxazole, cefoxitin, and gentamicin (Table IV). The probability of observing resistance to 4 of the drugs was significantly higher in isolates from nursery pigs than from grow-finish pigs and resistance to tetracycline and streptomycin was more likely in *Salmonella* from nursery pigs than from sows (Table IV). The odds of resistance to a given drug were not significantly different between *Salmonella* from grow-finish pigs and sows.

Overall, 59% (95% CI, 42 to 75) of the *Salmonella* were pansusceptible. Most isolates from sows (74%; 95% CI, 53 to 88) were pansusceptible, as were approximately half of the isolates from grow-finish (56%; 95% CI, 36 to 73) and nursery pigs (48%; 95% CI, 26 to 71). The odds of an isolate being pansusceptible were significantly higher in sows compared to the other production phases (Table V). Multiresistance was observed in 29% (95% CI, 16 to 48) of the isolates. The prevalence of multiresistance was higher in isolates from nursery pigs (48%; 95% CI, 26 to 71) than from grow-finish pigs (26%; 95% CI, 13 to 46) or sows (22%; 95% CI, 10 to 42). The difference between multiresistance in *Salmonella* from nursery pigs and sows was statistically significant (Table V).

Twenty-nine resistance patterns were identified and 9 occurred in more than 5 isolates (Table VI). Eight of these 9 were found in more than 1 phase in a herd. Despite this, isolates with the same pattern, from the same phase, and same herd, were not always the same serovar. Unobserved resistance patterns were also notable; resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT), suggestive of a chromosomally located gene cluster (37), was not identified in any isolate. Also noteworthy, the 13 *Salmonella* Typhimurium var. Copenhagen phage type 104 isolates were all pansusceptible.

Herd size was associated with resistance to streptomycin; the odds of resistance increased 1.02 times (95% CI, 1.01 to 1.03; $P = 0.003$) for every 1000 pigs marketed annually. No other resistance outcome was significantly associated with herd size ($P > 0.05$). Sample type was a significant predictor of 2 resistance outcomes. The odds of resistance

Table I. Population average prevalence estimates and 95% confidence intervals for resistance to each drug with minimum inhibitory concentrations (MIC) distributions of 468 *Salmonella* isolates

Antimicrobial	Prevalence	95% CI	Distribution of isolates (count) across dilution ranges															
			≤ 0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	> 512
Tetracycline	34.5	19.4–53.6									329	1					138	
Sulfamethoxazole	26.7	13.3–46.3									36	80	193	45			1	113
Streptomycin	13.6	7.5–23.3										413	27	28				
Ampicillin	12.6	3.4–36.7							263	60	72	5	6	1	61			
Kanamycin	10.2	2.6–33.0										413					55	
Chloramphenicol	5.0	1.6–14.3							1	60	265	111	11	20				
Trimethoprim-sulfamethoxazole	2.7	0.6–11.2					363	83	1	3	2	16						
Cephalothin	1.2	0.5–3.4								134	208	83	37	6				
Cefoxitin	0.6	0.2–2.2							2	137	207	45	74	3				
Gentamicin	0.2	0.0–1.7					382	48	37				1					
Amikacin	0	0.0–0.6						73	316	73	5	1						
Amoxicillin-clavulanic acid	0	0.0–0.6							315	90	2	54	7					
Ceftiofur	0	0.0–0.6					1	191	200	76								
Ceftriaxone	0	0.0–0.6					467			1								
Ciprofloxacin	0	0.0–0.6	379	50	38	1												
Nalidixic acid	0	0.0–0.6								41	343	81	3					

Shaded fields indicate the range tested for each antimicrobial and counts outside of shaded cells indicate isolates with growth in all wells within the tested range. For these isolates the actual MIC is greater than that range of dilutions. Vertical double bars mark the breakpoint between sensitive and resistant. Bold font indicates the median MIC.

Table II. Frequency of the 9 most common *Salmonella* serovars, the number of isolates per serovar with any resistance and number of isolates resistant to each antimicrobial (n = 468)

<i>Salmonella</i> Serovar (any resistance/ all isolates)	Number of isolates from each serovar resistant to each antimicrobial									
	AMP	CEP	CHL	COT	FOX	GEN	KAN	STR	SMX	TET
Derby (56/124)	—	4	22	12	3	—	—	17	24	31
Typhimurium var. Copenhagen (65/81)	58	2	2	—	—	—	54	9	58	61
Putten (2/49)	—	—	1	1	—	—	—	1	2	2
Infantis (0/37)	—	—	—	—	—	—	—	—	—	—
I:ROUGH-O:d:l,w (3/34)	—	—	—	2	—	—	—	1	3	2
Banana (0/27)	—	—	—	—	—	—	—	—	—	—
Mbandaka (24/26)	1	—	—	—	—	—	—	19	12	24
Anatum (1/20)	—	—	—	—	—	—	—	1	1	1
Give (3/19)	—	—	1	—	—	—	—	—	3	—
All others (21/51)	3	—	5	3	—	1	1	7	11	17

AMP — ampicillin, CEP — cephalothin, CHL — chloramphenicol, COT — trimethoprim-sulfamethoxazole, FOX — cefoxitin, GEN — gentamicin, KAN — kanamycin, STR — streptomycin, SMX — sulfamethoxazole, TET — tetracycline.

Table III. Distribution of production phases from which 1394 samples were collected, 452 *Salmonella* positive samples were identified and 468 *Salmonella* isolates were harvested

Production phase	Sample type	Samples collected	<i>Salmonella</i> positive	Isolates harvested
Nursery	PPS	255	81	83
Grow-finish	Individual	295	73	80
	PPS	545	158	161
Sow	Individual	100	38	38
	PPS	199	102	106
Total		1394	452	468

PPS — pooled pen sample.

to kanamycin were 1.2 times higher (95% CI, 1.1 to 1.3; $P < 0.0001$) in isolates from individual animals compared to PPS. In contrast, the odds of streptomycin resistance were decreased 0.47 times (95% CI, 0.26 to 0.86; $P = 0.02$) in samples collected from individual animals. Resistance to ampicillin, chloramphenicol, sulfamethoxazole, and tetracycline were not significantly associated with sample type ($P > 0.1$). Finally, knowledge of herd *Salmonella* status and laboratory performing isolation were not significantly associated with resistance to streptomycin, sulfamethoxazole, or tetracycline ($P > 0.2$). For resistance to ampicillin, chloramphenicol, and kanamycin, the significance of these variables could not be determined because of strong clustering within serovar.

Discussion

Describing AMR in *Salmonella* from on-farm studies provides insight into the epidemiology of resistance in pigs prior to transport, slaughter, and processing, which can affect *Salmonella* serovars and resistance (16,38,39). Different rates of resistance in each production phase shows *Salmonella* from each pig-class may pose unique food safety risks and suggests that resistance is dynamic within barns.

Table IV. Percent of *Salmonella* isolates from nursery pigs (n = 83), grow-finish pigs (n = 241), and sows (n = 144) resistant to each drug

Antimicrobial	Percent resistant		
	Nursery	Grow-finish	Sow
Tetracycline ^b	47.0	27.4	22.9
Sulfamethoxazole	41.0	16.6	27.8
Streptomycin ^{a,b}	22.9	8.7	10.4
Ampicillin ^a	19.3	9.1	16.7
Kanamycin ^a	16.9	7.9	15.3
Chloramphenicol	7.2	7.1	5.6
Trimethoprim-sulfamethoxazole	2.4	3.7	4.9
Cephalothin ^a	2.4	0.8	1.4
Cefoxitin ^c	0.0	0.4	1.4
Gentamicin ^c	0.0	0.4	0.0

^a Significant difference between isolates from nursery and grow-finish pigs ($P < 0.05$).

^b Significant difference between isolates from nursery pigs and sows ($P < 0.05$).

^c Models did not converge because frequency of resistance was too low.

Hence, future on-farm studies should investigate risk factors for resistance in each production phase. Identifying variables associated with changes in resistance between phases might lead to interventions to mitigate AMR in *Salmonella*.

Fluoroquinolones are used to treat invasive salmonellosis, while third-generation cephalosporins are indicated for *Salmonella* infections in children. There are few therapeutic alternatives to these antimicrobial classes (12,40). Because humans generally acquire resistant *Salmonella*, rather than the resistance developing during treatment, resistance to these drugs in isolates from food animals is of utmost importance (12). Like other North American reports, no resistance was noted to ceftriaxone, ciprofloxacin, or nalidixic acid (15,17,41,42).

Table V. Unconditional odds ratios for *Salmonella* pansusceptibility and multiresistance between isolates from each production phases (n = 468)

Outcome	Risk factor Source of <i>Salmonella</i>	Odds ratio	95%	
			Confidence interval	P
Pansusceptible ^a	Sow compared with nursery	3.0	1.2–7.8	0.02
	Sow compared with grow-finish	2.3	1.1–4.6	0.02
	Grow-finish compared with nursery	1.3	0.7–2.5	0.37
Multiresistant ^b	Nursery compared with sow	3.2	1.1–9.1	0.03
	Nursery compared with grow-finish	2.6	1.0–7.0	0.05
	Grow-finish compared with sow	1.2	0.8–1.9	0.36

^a Pansusceptible defined as susceptible to all 16 drugs considered.

^b Multiresistant defined as resistant to 2 or more of the 16 drugs considered.

Table VI. *Salmonella* spp. resistance patterns observed in more than 5 isolates; number identified in each production phase, number of herds phenotype was identified in, and number of serovars with phenotype identified

Resistance pattern	Number of isolates			Number of herds	Number of serovars
	Nursery	Grow-finish	Sow		
AMP KAN SMX TET	11	15	19	2	1
TET	1	31	4	4	6
SMX STR TET	5	4	5	4	3
STR TET	6	8	0	2	3
CHL	0	6	1	3	2
CHL SMX STR TET	5	2	0	1	5
AMP KAN SMX STR TET	2	1	3	1	1
SMX TET	5	0	1	3	3
CHL SMX STR COT	0	3	3	1	1

AMP — ampicillin, CHL — chloramphenicol, COT — trimethoprim-sulfamethoxazole, KAN — kanamycin, SMX — sulfamethoxazole, STR — streptomycin, TET — tetracycline.

Likewise, no resistance was found to ceftiofur. This differs from a report of frequent resistance to ceftiofur on United States farms (21%) (42). This difference may be partially explained by serotype as over 75% of the ceftiofur resistant *Salmonella* were *Salmonella* Derby (42). Canadian swine abattoir surveillance has noted changes in the *Salmonella* serovar distribution over time (43). Therefore, although no resistance to ceftiofur was reported in this study, common ceftiofur resistance in the United States suggests Canadian authorities and the swine industry should be vigilant for emerging third-generation cephalosporin resistance in *Salmonella* from pigs.

Antimicrobial resistance studies typically focus on close-to-market animals; however, information on resistance within pig categories provides insight into AMR within farms. Lower rates of AMR in older pigs suggest that resistant *Salmonella* populations may fluctuate within barns. Investigating why *Salmonella* resistance declines as pigs age might identify on-farm interventions to exploit this phenomenon. In a study of 3 farms, Nollet et al (44) observed less resistance in *Salmonella* from sows compared to growing pigs, but did not corroborate our observation of increased resistance in nursery pigs. However, others have reported relatively more resistance in coliforms from young pigs (45,46). These reports speculate that young animals carry more resistant organisms due to increased antimicrobial exposure and physiological differences (45,46). Identifying

risk factors for resistance in nursery pigs is crucial; weaned pigs commonly receive antimicrobials, and in some instances are continuously exposed to drugs, which raises concerns about selection for resistance (19,47,48). The effect of this exposure on resistance should be investigated and accounted for in judicious use guidelines.

Describing how resistance in *Salmonella* relates to herd was complicated by the relationship with serovar. This study was too small to account for serovar in the statistical analyses. Antimicrobial resistance is known to be associated with serovar and our inability to account for this important confounder is a study weakness (35,49–51). Future studies will need a much larger sample size to address this concern. The descriptive statistics revealed that the most common resistance pattern in this study occurred exclusively in *Salmonella* Typhimurium var. Copenhagen, and only in 2 herds, although *Salmonella* Typhimurium var. Copenhagen occurred in 4 other herds without this pattern. Investigating the extent that these isolates are related might indicate if this resistance was due to the spread of a resistant clone or common selective pressures. In numerous situations, *Salmonella* with the same resistance pattern and serovar were found in different production phases of the same herd. Thus also describing how isolates from different production phases are related could improve the understanding of how resistance spreads and persists within farms. Regardless of how

these isolates are related, the apparent clustering of serovars and resistance patterns within herds gives perspective to the food safety risk from these *Salmonella*: multiresistant *Salmonella* Typhimurium var. Copenhagen were frequently identified, but the herds with this pattern produced less than 2% of the pigs marketed by study herds. This demonstrates why abattoir-based monitoring, which can sample farms proportionate to the pork supply, can better estimate the food-safety risk from resistant *Salmonella*.

The few associations identified between given resistances and herd characteristics suggest that the study design did not substantially bias the results. Herd size was associated with resistance to streptomycin, so by excluding herds with less than 100 sows, resistance to streptomycin might have been slightly overestimated. However, as most pigs in Saskatchewan and Alberta are raised on large farms, restricting this study to herds with more than 100 sows more realistically reflected the source of market pigs from these provinces (52). Identifying different odds of streptomycin and kanamycin resistance in *Salmonella* from "individual-pig" compared with pooled pen fecal samples could be a type I error, because serovar was not accounted for. The biological importance of these associations is also questionable, as for both kanamycin and streptomycin, the predicted resistance from either sample type lies within the confidence intervals for the overall prevalence estimate. Finally, culture methods can affect the serovars isolated, so using 3 isolation methodologies could have introduced bias into these data (35,49). However, AFLB and PDS used similar methods to culture 89% of the samples and "laboratory" was not associated with any given resistance outcome, indicating bias was minimal. Overall, the study design does not appear to have biased the results substantially.

In summary, the resistance of *Salmonella* isolates from swine farms in Alberta and Saskatchewan is described. A high frequency of pansusceptibility and no resistance to 6 of the 16 antimicrobials are encouraging findings. Even so, the extent of multiresistance in these isolates was a concern. Few on-farm studies have described AMR in *Salmonella* from all age categories of pigs (35,44). This project identified many future research needs. Age-specific risk factor studies are needed to investigate reasons for differences in resistance between production phases. Likewise, further description of the associations between resistance and serovar, and how resistance spreads within herds, are needed before effective intervention strategies can be designed to control AMR in *Salmonella* from pigs.

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