

# Describing the *Salmonella* classification levels for low-volume production systems utilizing abattoir-based samples and classification stability over time

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## Summary

**Objective:** To compare estimates of the prevalence of meat-juice-based antibodies to *Salmonella* in swine originating from low-volume production systems (marketing  $\leq$  8000 pigs per year) during 2002 and 2004.

**Materials and methods:** Results of testing meat-juice samples by a commercial indirect enzyme-linked immunosorbent assay (ELISA) were available for 2002 and 2004 for swine marketed by 502 low-volume swine-production systems through eight commercial Iowa abattoirs with high throughput ( $>$  1000 head per hour).

**Results:** In 2002, 934 of 14,401 samples (6.5%), and in 2004, 1639 of 13,718 samples (11.9%) were seropositive for

*Salmonella* (ELISA sample-to-positive ratio  $\geq$  0.4). Average *Salmonella* seroprevalence in 2002 was 6.8%, median prevalence was 0.8%, and within-producer prevalence range was 0% to 59.2%. In 2004, average seroprevalence was 11.8%, median prevalence was 5.2%, and range was 0% to 81.8%. In 47% of low-volume production systems classified according to the Danish *Salmonella* classification system, classification did not change from 2002 to 2004. However, 53% of systems did change classification, with most moving to classifications representing higher observed seroprevalence.

**Implications:** Population *Salmonella* seroprevalence is not stable within defined and matched swine-production cohorts over time. Within-herd *Salmonella* seroprevalence

is not stable in smaller production herds over time. These variations should be considered when making inferences about the risk of *Salmonella* in individual sites or swine-producing regions and for intervention programs that measure success by monitoring *Salmonella* seroprevalence at the production-system level. Classification of production-system status based on *Salmonella* antibody prevalence is an unstable outcome over time.

**Keywords:** swine, *Salmonella*, zoonosis, *Salmonella* seroprevalence, meat-juice survey

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## Resumen - Descripción de los niveles de clasificación de *Salmonella* para sistemas de producción de bajo volumen que utilizan muestras de mataderos y la clasificación de estabilidad al paso del tiempo

**Objetivo:** Comparar los cálculos de prevalencia de los anticuerpos contra *Salmonella* a partir de exudados de carne en cerdos originados de sistemas de bajo volumen de producción (vendiendo  $\leq$  8000 cerdos por año) durante 2002 y 2004.

**Materiales y métodos:** Los resultados de muestras de exudado de carne realizados con una prueba comercial de enzimoanálisis de adsorción indirecta (ELISA)

estuvieron disponibles para los cerdos vendidos en 2002 y 2004 por 502 sistemas de producción de cerdo de bajo volumen en ocho mataderos comerciales de alto rendimiento ( $>$  1000 cabezas por hora) de Iowa.

**Resultados:** En 2002, 934 de 14,401 muestras (6.5%), y en 2004, 1639 de 13,718 muestras (11.9%) resultaron seropositivas a *Salmonella* (ELISA muestra-a-resultado positivo  $\geq$  0.4). La seroprevalencia de *Salmonella* en 2002 fue de 6.8%, la prevalencia mediana fue de 0.8%, y la prevalencia entre productores tuvo un rango de 0% a 59.2%. En 2004, la seroprevalencia promedio fue de 11.8%,

la prevalencia mediana fue de 5.2%, y el rango fue de 0% a 81.8%. En 47% de los sistemas de producción de bajo volumen clasificados de acuerdo a los sistemas de clasificación Danesa para *Salmonella*, la clasificación no cambió de 2002 a 2004. Sin embargo, 53% de los sistemas sí cambiaron de clasificación, la mayoría cambiando a clasificaciones que representaban una seroprevalencia observada más alta.

**Implicaciones:** La seroprevalencia de *Salmonella* de la población dentro de cohortes definidos de cerdos no es estable al paso del tiempo. La seroprevalencia de *Salmonella* dentro del hato en sistemas de producción pequeños no es estable al paso del tiempo. Estas variaciones deberían considerarse al hacer inferencias sobre el riesgo de *Salmonella* en sitios individuales o en regiones de producción de cerdos y para programas de intervención que miden el éxito monitoreando la seroprevalencia de *Salmonella* a nivel del sistema de producción. La clasificación del estatus del sistema de producción basada en la prevalencia de anticuerpos de *Salmonella* ofrece un resultado inestable al paso del tiempo.

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## Résumé - Description des niveaux de classement pour *Salmonella* de systèmes de production à faible volume au moyen d'échantillons prélevés à l'abattoir et stabilité de la classification dans le temps

**Objectif:** Comparer les estimations de la prévalence d'anticorps contre *Salmonella* dans le jus de viande chez des porcs provenant d'organisation à faible volume de production (mise en marché de  $\leq 8000$  porcs par année) durant les années 2002 et 2004.

**Matériels et méthodes:** Les résultats d'analyse du jus de viande par un essai immuno-enzymatique indirect (ELISA) commercial étaient disponibles pour 2002 et 2004 pour des porcs mis en marché par 502 systèmes de production à faible volume via huit abattoirs commerciaux

en Iowa qui avaient une vitesse élevée d'abattage ( $> 1000$  têtes par heure).

**Résultats:** En 2002, 934 des 14,401 échantillons (6.5%), et en 2004, 1639 des 13,718 échantillons (11.9%) étaient positifs pour *Salmonella* (ratio échantillon-à-positif par ELISA  $\geq 0.4$ ). La séroprévalence moyenne pour *Salmonella* en 2002 était de 6.8%, la prévalence médiane était de 0.8%, et l'étendue de la prévalence à l'intérieur d'une production allait de 0% à 59.2%. En 2004, la séroprévalence moyenne était de 11.8%, la prévalence médiane de 5.2%, et l'étendue de 0% à 81.8%. Pour 47% des systèmes de production à faible volume classifiés selon les systèmes de classification danois, la classification est demeurée inchangée entre 2002 et 2004. Toutefois, 53% des systèmes ont changé de classification, la plupart changeant à des classifications

représentant des séroprévalences observées plus élevées.

**Implications:** La séroprévalence de *Salmonella* dans le temps dans une population n'est pas stable à l'intérieur de cohortes de production porcine définies et appariées. Dans le temps, la séroprévalence à l'intérieur d'un troupeau n'est pas plus stable dans des troupeaux à plus faibles productions. Ces variations devraient être prises en considération lorsque des inférences sont faites sur le risque associé à *Salmonella* sur des sites individuels ou des régions de production porcine et pour les programmes d'intervention qui évaluent les succès en surveillant la séroprévalence de *Salmonella* au niveau du système de production. La classification du statu d'un système de production basée sur la prévalence d'anticorps contre *Salmonella* est un résultat instable dans le temps.

In 1995, the Danish swine industry launched the first on-farm *Salmonella* control program to categorize production sites as to their risks for *Salmonella* contamination.<sup>1</sup> The Danish national program has undergone several iterations, but the concept of production-site risk categorization has remained a constant. Similar programs are being implemented in other European Union countries and also in Quebec, Canada. *Salmonella* classifications have been used to schedule transport and to harvest herds of similar status to reduce antemortem *Salmonella* cross-contamination or as motivation for on-farm interventions. No systematic classification of United States production systems by *Salmonella* seroprevalence has been attempted. This paucity of information hampers the ability to conduct farm-to-abattoir risk assessments and to determine the feasibility of on-farm control programs as a method to reduce carcass *Salmonella* contamination. However, although the national or regional prevalence of *Salmonella* classes could be used to describe a region's *Salmonella* status and to assess the utility of interventions directed at the farm, factors other than the intervention that cause changes in the classifications must be understood to create a pathway to action. The first step in understanding sources of variation is determining whether meaningful fluctuations actually occur or if the estimates are stable. Therefore, the aim of the study was to evaluate the stability of the Danish *Salmonella* herd-classification status applied to herds that were not subject to formal *Salmonella* control programs and to determine

the percentage of herds that remain in the same category over time.

## Materials and methods

Test results of meat-juice samples derived by a commercial indirect enzyme-linked immunosorbent *Salmonella* antibody assay (ELISA) obtained from swine marketed by 502 low-volume swine-production systems ( $\leq 8000$  head annually) in both 2002 and 2004 were presented for analysis. In 2002 and 2004, diaphragm samples were collected from swine slaughtered at eight high-throughput commercial Iowa abattoirs ( $> 1000$  head per hour).<sup>2</sup> The samples used in the study were a subset of samples collected as part of the Iowa pseudorabies monitoring program. In 2002, four diaphragm samples were selected by convenience from each study lot during processing. In 2004, two diaphragm samples were similarly collected from each study lot. For each study period, diaphragm samples were collected daily for 3 months. After collection, the samples were uniquely identified by lot number, date, and abattoir, and at the end of the collection period were matched by this identity to a production system.

For both time periods 2002 and 2004, after sample reception, meat juice was decanted from the diaphragm samples following a freeze-thaw cycle, and subsequently submitted to the Iowa State University Veterinary Diagnostic Laboratory for analysis using a commercially available indirect *Salmonella* ELISA (HerdChek *Salmonella* ELISA; Idexx Laboratories Inc,

Westbrook, Maine). For each meat-juice sample, the result was reported as a sample-to-positive (S:P) ratio and converted to a dichotomous outcome, positive for results  $\geq 0.4$  and negative for results  $< 0.4$ , based upon corresponding serum cut-off values supplied by the company.

Production systems were classified into the Danish classification on the basis of cumulative *Salmonella* seroprevalence for each 3-month period in both 2002 and 2004 data. The levels are comparable to classifications used by the Danish system, Levels 0, 1, 2, and 3, which have been described by Mousing et al<sup>3</sup> and Alban et al.<sup>4</sup> The cut-off points for each level differed by annual estimated swine marketing (Table 1), as an example, for production systems marketing between 201 to 500 swine per year, Level 0 was no or negligible evidence of *Salmonella* exposure, and Level 1 was defined as systems with *Salmonella* antibodies detected in  $> 0\%$  to 25% of swine. However, for systems marketing 3001 to 8000 swine per year, Level 1 was defined as systems with antibody prevalence  $> 0\%$  to 17%.

To evaluate changes in each production system's *Salmonella* classification from 2002 to 2004, the difference in classification was calculated by subtracting the 2002 classification from the 2004 classification, eg, a production system in Level 1 in 2002 and Level 3 in 2004 was described as having a +2 increase in *Salmonella* classification level change ( $3 - 1 = +2$ ), as would a production system in Level 0 in 2002 and Level 2 in 2004 ( $2 - 0 = +2$ ). A production system in Level 3 in 2002

**Table 1:** Seroprevalence cut-off points to categorize herds for past exposure to *Salmonella* when meat-juice from diaphragm samples collected at slaughter were tested serologically\*

Estimated annual harvest	Seroprevalence cut-off point (%)			
	Negative	Level 1	Level 2	Level 3
≤ 200	0	> 0 - 50	NA	> 50
201-500	0	> 0 - 25	> 25 - 50	> 50
501-1000	0	> 0 - 23	> 23 - 50	> 50
1001-2000	0	> 0 - 20	> 20 - 50	> 50
2001-3000	0	> 0 - 17	> 17 - 50	> 50
3001-5000	0	> 0 - 17	> 17 - 50	> 50
> 5000†	0	> 0 - 17	> 17 - 33	> 33

\* Meat-juice samples were tested using an indirect *Salmonella* enzyme-linked immunosorbent assay (HerdChek *Salmonella* ELISA; Idexx Laboratories Inc, Westbrook, Maine). Samples with sample-to-positive ratio < 0.4 were considered negative.

† A herd was assigned to Level 3 if prevalence of positive samples (sample-to-positive ratio ≥ 0.4) was > 33%.<sup>3</sup>

NA = not applicable.

and Level 2 in 2004 would be described as a -1 level change (2 - 3 = -1), as would a production system in Level 1 in 2002 and Level 0 in 2004 (0 - 1 = -1). To test the null hypothesis that the distribution of low-volume production systems within the Danish *Salmonella* classification Levels 0, 1, 2, and 3 was not different in 2002 as 2004, a chi-squared test for proportions was used (using R software, 2008 version, available at [www.R-project.org](http://www.R-project.org)).

An ancillary analysis conducted evaluated whether the proportions of low-volume and high-volume (> 25,000 head marketed annually) production systems in each of the Danish *Salmonella* classification Levels 0, 1, 2, and 3 were the same in 2002 and 2004. This hypothesis was tested using a chi-squared test for proportions using R software, 2008 version.

## Results

In 2002 and 2004, respectively, a total of 14,401 and 13,718 meat-juice samples were collected. In 2002, 934 samples (6.5%) were seropositive for *Salmonella*; in 2004, 1639 samples (11.9%) were seropositive ( $P < .01$ ). Samples were collected from 1088 production systems in 2002 and 919 in 2004, with data from 794 production systems available for both periods. After excluding data from production systems with < 12 samples collected over the 3 months in the 2002 and 2004 sampling periods, 502 low-volume production systems were available for the

analysis comparing 2002 to 2004 *Salmonella* classification levels. In 2002, 45 high-volume production systems were available and in 2004, 87 high-volume production

systems were available. It was not possible to match 2004 data with 2002 data for high-volume production systems, ie, the matched comparison was not conducted for high-volume production systems.

For the 502 low-volume production systems in 2002, average production-system *Salmonella* seroprevalence was 6.38%, median production-system seroprevalence was 0.8%, and within-production system seroprevalence range was 0% to 59.2%. In 2004, average production-system *Salmonella* seroprevalence was 11.8%, median seroprevalence was 5.2%, and range was 0% to 81.8%. *Salmonella* seroprevalence was higher in 2004 than in 2002 (chi-squared test;  $P < .01$ ). Table 2 shows average prevalence of *Salmonella* for each low-volume production system, based on the estimated annual slaughter. In Table 3, the percentage of low-volume production systems in the *Salmonella* classification levels in 2002 and 2004 are presented; these proportions were significantly different (chi-squared test;  $P < .01$ ). Table 4 demonstrates the changes in classification for producers between 2002 and 2004. For example, 240

**Table 2:** Distribution of the within-herd *Salmonella* seroprevalence by annualized production estimates for 502 low-volume production systems\* in 2002 and 2004

Herd size	Frequency	Mean (%) (95% CI)	Median (%)	Range (%)
<b>Year 2002</b>				
≤ 200	2	NA	NA	NA
201-500	56	4.5 (1.8-7.3)	0	0
501-1000	159	6.1 (4.4-7.8)	0	59.2
1001-2000	168	6.8 (5.2-8.4)	2.8	53.3
2001-3000	66	8.9 (5.8-12.1)	3.5	54.5
3001-5000	48	4.6 (2.6-6.6)	2.6	37.0
5001-8000	3	1.5	2.3	2.4
<b>Year 2004</b>				
≤ 200	2	NA	NA	NA
201-500	56	8.7 (4.2-13.3)	0	68.7
501-1000	159	11.2 (8.5-13.8)	4.2	69.2
1001-2000	168	11.8 (9.2-14.3)	6.2	80
2001-3000	66	15.1 (10.9-19.1)	9.2	70.5
3001-5000	48	13.3 (7.4-19.7)	4.5	81.8
5001-8000	3	19.7	22.8	25.0

\* Low-volume production systems marketed ≤ 8000 swine annually. Meat juice obtained from diaphragm samples collected at slaughter was tested.

NA = not applicable. In 2002, prevalence was 0% on both farms; in 2004, prevalence was 0% on one farm and 7.1% on the other.

**Table 3:** Frequency distribution of categories describing evidence of past exposure to *Salmonella* in the same 502 low-volume production systems that marketed swine in Iowa in 2002 and 2004\*

Year of collection	No. of herds	Frequency (%)†			
		Negative	Level 1	Level 2	Level 3
2002	502	251 (50)	207 (41)	40 (8)	4 (1)
2004	502	172 (34)	230 (45)	71 (14)	29 (6)

\* Low-volume production systems marketed  $\leq 8000$  swine annually. Diaphragm samples were collected at abattoirs; in 2002, four samples were collected from each study lot and in 2004, two samples were collected from each study lot. Meat juice obtained was tested using a commercially available indirect *Salmonella* ELISA (HerdChek *Salmonella* ELISA; Idexx Laboratories Inc, Westbrook, Maine). Results were reported as sample-to-positive ratios, with results  $\geq 0.4$  considered positive. The proportions of herds in the *Salmonella* classification levels were significantly different in 2002 and 2004 (chi-squared test;  $P < .01$ ).

† *Salmonella* classification levels described in Table 1.

**Table 4:** Change in *Salmonella* level\* in 2004 compared to the level observed in 2002 for 502 low-volume production systems marketing swine in Iowa

	Change in classification level					
	-2	-1	0	1	2	3
No. of herds (%)	8 (1.6)	67 (13.3)	240 (47.8)	138 (27.5)	42 (8.4)	7 (1.4)

\* Classification levels are described in Table 1. A production system in Level 1 in 2002 and Level 3 in 2004 would be described by a +2 ( $3 - 1 = +2$ ) level change, as would production system in Level 0 in 2002 and Level 2 in 2004 ( $2 - 0 = 2$ ). A production system in Level 3 in 2002 and Level 2 in 2004 would be described as a -1 ( $2 - 3 = -1$ ) level change, as would a production system in Level 1 in 2002 and Level 0 in 2004 ( $0 - 1 = -1$ ).

of 502 low-volume production systems (47.8%) showed no difference in the category in 2004 compared to the 2002 estimate, whereas the remaining 262 systems (52.2%) changed classifications, with 187 low-volume production systems (37.3%) reported in higher levels in 2004 compared to 2002. The cross tabulation in Figure 1 further demonstrates the changes in the *Salmonella* classification levels between 2002 and 2004 for low-volume production systems. No low-volume production systems classified as Level 3 in 2002 were still classified as Level 3 in 2004. Figure 2 shows the scatter plot of seroprevalence for each production system in 2002 and 2004. If production systems maintained a similar seroprevalence, then the data should resemble dots clustered along a straight line. Data were also available from several high-volume producers, and these data are reported in Table 5. Eighty-seven high-volume production systems were surveyed in 2004 compared to the 45 high-volume production systems in 2002.

The ancillary analysis provided evidence for rejecting the null hypothesis that the

distribution of low-volume production systems across the Danish classification levels was the same as the distribution of the high-volume production systems in 2002 ( $P < .01$ ) and again in 2004 ( $P < .01$ ).

## Discussion

Comparison of 2002 and 2004 herd *Salmonella* seroprevalence data for low-volume production systems was the primary objective

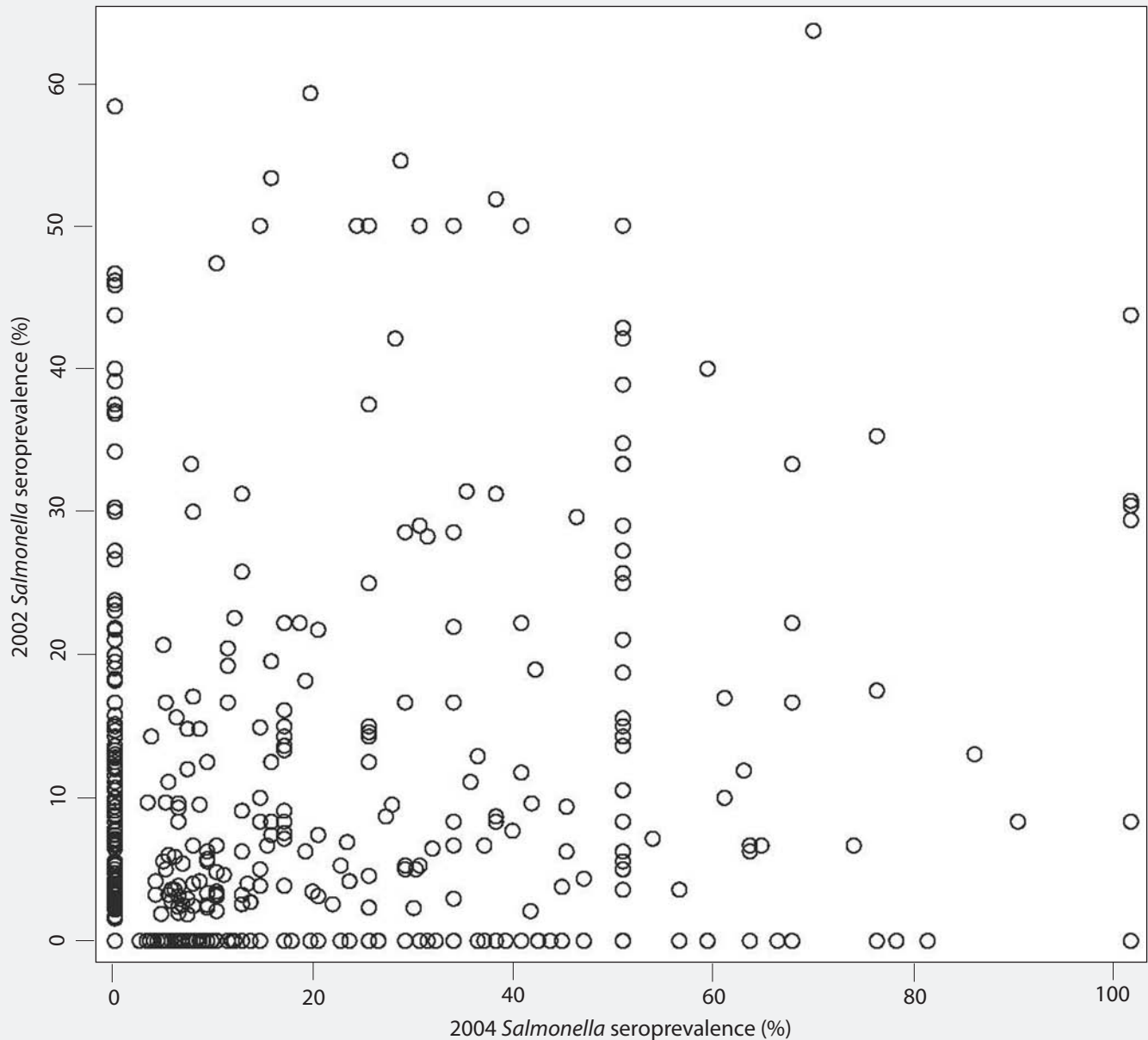
of this study. *Salmonella* seroprevalence was higher in 2004 (11.9%) than in 2002 (6.5%), suggesting that seroprevalence was not stable between 2002 and 2004 among low-volume production systems and that the prevalence of *Salmonella* exposure in Midwestern swine differed between the two surveys. The results highlight that Danish classifications assigned to production systems in 2002 were not likely to be the same in 2004. This finding has implications for *Salmonella* control schemes that use antibody-prevalence classification criteria to impose marketing restriction on herds.

One reason for the change in seroprevalence from year to year may be natural variation in the ecology of *Salmonella*; however, the causes of variability in *Salmonella* prevalence within a production system or swine population over time are not well understood. Considering the large number and diversity of smaller-volume sites compared in this study, *Salmonella* seroprevalence fluctuations over time

**Figure 1:** Comparative prevalence distribution for *Salmonella* classifications of low-volume swine production systems (marketing  $\leq 8000$  swine per year) in 2002 and 2004. Meat-juice samples collected at eight Iowa abattoirs were tested using a commercially available indirect *Salmonella* enzyme-linked immunosorbent assay (HerdChek *Salmonella* ELISA; Idexx Laboratories Inc, Westbrook, Maine). Classification levels described in Table 1. White font indicates the same classification in 2002 and 2004.

Year	2004				
	Negative	Level 1	Level 2	Level 3	Total
2002	Negative	Level 1	Level 2	Level 3	Total
Negative	117 (46.6%)	100 (39.8%)	27 (10.8%)	7 (2.8%)	251
Level 1	49 (23.7%)	112 (54.1%)	31 (15.0%)	15 (7.2%)	207
Level 2	6 (15.0%)	16 (40.0%)	11 (27.5%)	7 (17.5%)	40
Level 3	0	2 (50.0%)	2 (50.0%)	0	4
<b>Total</b>	<b>172</b>	<b>230</b>	<b>71</b>	<b>29</b>	<b>502</b>

**Figure 2:** Scatter plot of *Salmonella* seroprevalence in 2002 and 2004 for 502 low-volume swine production systems (marketing  $\leq 8000$  swine per year). Meat-juice samples collected at eight Iowa abattoirs were tested using a commercially available indirect *Salmonella* enzyme-linked immunosorbent assay (HerdChek *Salmonella* ELISA; Idexx Laboratories Inc, Westbrook, Maine). For each meat-juice sample, the result was reported as a sample-to-positive ratio, with results  $\geq 0.4$  considered positive and results  $< 0.4$  considered negative.



within and between sites are unlikely to be due to significant changes in management or system configurations. The results of the ancillary analysis and descriptive data suggest that larger-volume production systems were associated with higher *Salmonella* seroprevalence classification than smaller units, irrespective of the sampling period. If these observations hold true for populations beyond the study population, the cause(s) of that variation should be understood further before *Salmonella* seroprevalence classifications are used to impose market restrictions on production systems.

The Danish classification scheme utilizes within-herd antibody prevalence values to place herds in appropriate risk designations. With a near doubling of the seroprevalence from 6.5% in 2002 to 11.9% in 2004, the 502 low-production systems compared by size demonstrated a significant rise of within-herd prevalences for herd sizes 501-1000, 1001-2000, 2001-3000, and 3001-5000. Although 47.8% of the low-volume production systems did not change level status, those that did change tended to move to higher classification ie, 187 of 262 of low-volume

production systems (71.4%) that changed status showed an increase in *Salmonella* seroprevalence in the swine marketed by the system. An explanation of these variations within the total population, although not surprising given the Danish experiences, is not readily apparent. The absence of more than two observation points makes further evaluation of these changes problematic. Additional sampling under similar configurations would demonstrate whether this increase is sustainable or represents an aberration.

**Table 5:** Prevalence frequency distribution of categories describing evidence of past exposure to *Salmonella* in high-volume swine production systems for 2002 and 2004\*

Year sampled	No. of herds	Frequency (%)			
		Negative	Level 1	Level 2	Level 3
2002	45	5 (11)	27 (60)	12 (27)	1 (2)
2004	87	3 (3)	59 (68)	22 (25)	3 (3)

\* High-volume production systems marketed > 25,000 swine annually. Collection and testing of samples described in Table 3. Classification levels described in Table 1.

Another possible explanation for the change in *Salmonella* classification level may be in use of different sampling algorithms in 2002 and 2004 because of modifications in the pseudorabies monitoring program. The sampling rate was four carcasses per lot in 2002 and two carcasses per lot in 2004. The expected direction of bias associated with collecting fewer samples should be less detection of *Salmonella* and lower *Salmonella* seroprevalence in 2004; however, such an outcome was not observed. Instead, higher *Salmonella* seroprevalence was observed, suggesting that, if bias did occur due to a lower sampling rate, the observed 37.3% increase in herds with higher classifications is a conservative estimate. Another source of bias may be a selective loss of production systems between 2002 and 2004. Unfortunately, 417 low-volume producers identified in 2002 were lost by 2004. It is likely, given the dynamics of swine production during this period, that many of these systems stopped production and were differentially in the smaller annual-production classifications. Matching by production-system size and reporting results only from production systems sampled in both time periods should have eliminated the potential for this bias.

Therefore, such within-herd serologic variability should be considered when making inferences about the risk of *Salmonella* in individual sites or swine-producing regions. Intervention programs that measure decreased risk by monitoring *Salmonella* seroprevalence at the production-system level should be accompanied by comparative data at harvest to assure that the effects are realized in the post-harvest environment. Otherwise, the salutary effects believed to accrue from classification and management practices to maintain a low herd-prevalence status may not be passed from production to the consumer.

### Implications

- Population *Salmonella* seroprevalence is not stable within defined and matched swine-production cohorts over time.
- Within-herd *Salmonella* seroprevalence is not stable in smaller production herds over time.

- These variations should be considered when making inferences about the risk of *Salmonella* exposures in individual sites or swine-producing regions and for intervention programs that measure success by monitoring *Salmonella* seroprevalence at the production-system level.

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