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Effect of time in lairage on caecal and carcass salmonella contamination of slaughter pigs

BY I. R. MORGAN, F. L. KRAUTIL AND J. A. CRAVEN
Attwood Veterinary Research Laboratory, Mickleham Road, Westmeadows, Victoria, Australia 3047

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SUMMARY

A longitudinal study of the effect of time spent in lairage on salmonellas in the caecum and on the skin surface of 450 slaughter pigs from a single producer was conducted. Pigs were tested in 6 groups at 2 abattoirs, with one-third of a group being slaughtered after 18 h, one-third after 42 h and one-third after 66 h spent in lairage. The salmonella isolation rate from caeca and carcass surfaces increased significantly with increasing time spent in lairage. Salmonellas were isolated from the caeca of 18·5 % of pigs held less than 24 h in lairage, 24·1 % of pigs held a further 24 h and 47·7 % of pigs held for 66 h in lairage before slaughter. The salmonella isolation rates from carcasses were 9·3 %, 12·8 % and 27·3 % for the same groups. Thirteen salmonella serotypes were isolated from the caecal contents and carcasses over the 6 weeks of the trial. One abattoir had a higher salmonella isolation rate from pigs than the other and this was probably related to lairage management. It appeared that lairage is an important factor in the manipulation of the salmonella contamination of pig carcasses.

INTRODUCTION

A number of studies have shown that pre-slaughter handling of pigs can affect the salmonella isolation rate in slaughter pigs. Transport time, feeding, environmental contamination and length of time in lairage all may affect the salmonella isolation rate (Williams & Newell, 1968; Shotts, Martin & Galton, 1962; Childers, Keahey & Kotula, 1977; Hansen *et al.* 1964; Craven & Hurst, 1982). Most studies reported that the proportion of pigs carrying salmonella in the caeca was directly related to the length of stay in lairage at abattoirs. In contrast, Craven & Hurst (1982) obtained clear evidence of a fall in the proportion of pigs with caecal salmonella when they were held in lairage for up to 72 h. The proportion of pigs with caecal salmonellas in the latter study was high on arrival at the abattoir. This study was also longitudinal in that the same group of pigs, randomly allocated to lairage groups, were used to examine the effect of lairage on caecal salmonellas. Most other studies have used different groups of pigs, assuming that there is a general level of caecal salmonella infection in pigs entering abattoirs. These studies also compared the isolation rate of salmonellas from rectal faeces on arrival at the abattoir with subsequent isolation rates from caecal contents. There are a number

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of problems with this approach as rectal isolation is usually inefficient at detecting salmonellas in pigs (Haddock, 1970).

This paper reports the results of a longitudinal study of the effect of time spent in lairage on salmonellas in the caecum and on the carcass of pigs.

MATERIALS AND METHODS

Pigs

The pigs examined in this study were derived from a single producer. For each experiment 75 pigs from the same production unit on the farm were used. The pigs arrived at the abattoir at approximately 1 p.m. after leaving the farm at 8 a.m. that morning and having travelled approximately 300 km. A group of 75 were drafted from the truck load and tattooed to identify them during processing. At Abattoir A, 25 pigs were selected at random and placed in a small holding pen near the slaughter floor, the remaining 50 pigs were held in a large pen in lairage. At Abattoir F, the 75 pigs were randomly allocated to 3 groups of 25 and kept in separate holding pens. Groups of pigs were killed on Monday, Tuesday and Wednesday mornings and were the first, or among the first, pigs processed for the day. Pigs were thus killed after 18, 42 and 66 h in lairage. Not all slaughter groups contained 25 pigs due to occasional loss of identification and escape of pigs from groups.

The pigs held 42 and 66 h were fed with a commercial pig ration placed on the floor once per day. Water was available at all times.

Pen hygiene was markedly better at Abattoir F with the pens being hosed daily.

The experiment was repeated three times at each of the abattoirs.

Sampling

Approximately 25 gram of caecal contents were collected from each pig after it was eviscerated. An opening was made in the caecum with a sterile scalpel blade and caecal contents were collected into individual sterile polypropylene containers. The samples were stored on ice for transport to the laboratory.

Swabs were collected from each carcass as it entered the chiller. These samples were collected by rubbing a large cotton-tipped swab over both sides of the carcass from the ham to the jowl. The swab was placed in 10ml of buffered peptone water and transported on ice to the laboratroy for processing.

Carcass weights

Carcass weights were collected for each pig to examine whether there were any effects on carcass weight from prolonged time in lairage.

Salmonella isolation procedure

Caecal contents. Swabs were used to transfer caecal samples into 10 ml mannitol selenite (MS) broth (Oxoid) containing 0·1 % L-cystine, and into 10 ml of tetrathionate broth (Oxoid) containing 1:100000 brilliant green (Tet-BG). In addition, approximately 0·1 ml of caecal contents was inoculated into 10 ml of Rappaport-Vassiliadis (RV) broth (Vassiliadis *et al.* 1981). The MS broth was incubated at 42 °C, the Tet-BG broth at 37 °C and the RV broth at 43 °C. After 18–24 h

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Table 1. *Isolation of salmonella from caecal contents and carcass surfaces of pigs held in lairage*

Expt.	Abbattoir	Caecal salmonella isolation rate Day 1	Day 2	Day 3	Carcass swab isolation rate Day 1	Day 2	Day 3
1	A	13/25	10/23	19/25	0/25	1/25	1/25
2	A	0/25	2/24	4/25	0/25	0/24	0/25
3	A	7/25	17/24	22/25	1/25	14/24	21/25
	Total A	20/75	29/71	45/75	1/75	15/73	22/75
4	F	3/25	0/25	25/25	13/25	1/25	19/25
5	F	1/25	1/25	0/24	0/25	3/25	0/25
6	F	4/26	5/24	1/25	0/25	0/25	0/25
	Total F	8/76	6/74	26/74	13/75	4/75	19/75
Combined results		28/151 (18·5%)	35/145 (24·1%)	71/149 (47·7%)	14/150 (9·3%)	19/148 (12·8%)	41/150 (27·3%)

incubation, each of the selective enrichment broths were plated onto xylose lysine desoxycholate (XLD) agar (Gibco) and Bismuth Sulphite (BS) agar (Gibco). The XLD plates were incubated for 24 h at 37 °C, and the BS plates for 48 h at 37 °C. After incubation the plates were examined and up to two suspect salmonellas colonies were picked from each plate and submitted to biochemical and serological identification procedures. The identity of all salmonella isolates was confirmed at the Microbiological Diagnostic Unit, University of Melbourne, Australia.

Carcass swabs. Each swab was stomached in an additional 90 ml of buffered peptone water with a Colworth Stomacher 400 (Seward Laboratories, London) for 30 sec. They were then incubated for 18 h at 37 °C. After incubation, 1 ml of the suspension was then inoculated into MS broth and into Tet-BG broth. An amount of 0·1 ml of the suspension was also inoculated into RV broth. The enrichment broths were then processed as described for isolation of salmonellas from the caecal contents.

Antibiotic sensitivity testing

Cultures were inoculated into 3·5 ml of double strength nutrient broth (Difco) and incubated for 90 min in a 37 °C shaking water bath. Approximately 30 μl was added to 3·5 ml of 1% peptone water, mixed and then approximately 30 μl was added to the wells of the Clements antibiotic sensitivity replicator tray (H. R. Clements P/L., Sydney, Australia) which contained 0·5 ml of nutrient broth with 0·05% agar. Seven cultures of known antibiotic resistance were included in each run. Using the Clements antibiotic sensitivity replicator (H. R. Clements P/L., Sydney, Australia) the cultures were spotted on lysed blood isosensitest agar (Oxoid) plates containing the appropriate antibiotics. Control plates of lysed blood isosensitest agar were incorporated at the beginning and the end of the run as well as an XLD and a MacConkey agar plate at the end of the run to check for purity of the cultures. Plates were incubated at 37 °C overnight then examined for growth (resistant) or no growth (sensitive).

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Abattoir A

Expt.	Day	Serotype	Resistance pattern	Isolates from: Caeca	Isolates from: Carcass
1	1	*S. derby*	-------	13	0
	2	*S. derby*	-------	10	0
		S. stanley	-------	0	1
	3	*S. derby*	-------	19	1
2	1	No isolates		—	—
	2	*S. bovis morbificans*	NNNNNNN	1	0
		S. untypable	-------	1	0
	3	*S. typhimurium*	-------	1	0
		S. 4.5.12:d	NNNNNNN	2	0
		S. agona	-------	1	0
3	1	*S. havana*	NNNNNNN	1	0
		S. havana	TET,KAN,STR,SUL	3	0
		S. typhimurium	NNNNNNN	1	0
		S. dublin	NNNNNNN	1	0
		S. agona	-------	1	0
		S. bredeney	-------	0	1
	2	*S. havana*	NNNNNNN	4	11
		S. havana	-------	1	3
		S. derby	NNNNNNN	1	0
		S. derby	-------	1	0
		S. give	NNNNNNN	7	0
		S. give	-------	1	0
		S. typhimurium	NNNNNNN	1	0
		S. worthington	-------	1	0
	3	*S. derby*	NNNNNNN	4	0
		S. derby	-------	1	0
		S. derby	STR	1	0
		S. give	NNNNNNN	4	0
		S. give	-------	2	0
		S. agona LD-	NNNNNNN	6	21
		S. agona LD-	KAN	2	0
		S. agona LD-	KAN,STR	2	0

Abbattoir F

Expt.	Day	Serotype	Resistance pattern	Isolates from: Caeca	Isolates from: Carcass
4	1	*S. anatum*	-------	1	0
		S. give	TET,KAN,STR,SUL,TRI	1	0
		S. give	-------	0	13
		S. bovis morbificans	-------	1	0
	2	*S. bredeney*	TET	0	1
	3	*S. anatum*	NNNNNNN	24	18
		S. anatum	-------	0	1
		S. anatum	STR	1	0
5	1	*S. bredeney*	NNNNNNN	1	0
	2	*S. bredeney*	TET,KAN,STR,SUL	1	0
		S. agona	-------	0	2
		S. agona	NNNNNNN	0	1
	3	No isolates		—	—
6	1	*S. agona*	-------	1	0
		S. havana	TET,KAN,STR,SUL	3	0
	2	*S. agona*	NNNNNNN	2	0
		S. havana	TET,KAN,STR,SUL	2	0
		S. infantis	TET,KAN,STR,SUL	1	0
	3	*S. agona*	-------	1	0

N, not tested: -------, sensitive to TET,CHL,KAN,STR,AMP,SUL,TRI.

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Table 3. *Mean carcass weights of slaughter pigs held in lairage*

	Mean weight of pigs (kg)					
	Held in lairage 18 h		Held in lairage 42 h		Held in lairage 66 h	
Experiment	Weight	S.E.	Weight	S.E.	Weight	S.E.
1	63·24	0·87	61·36	0·96	60·76	1·27
2	62·04	1·18	59·25	1·43	62·32	0·95
3	60·56	1·12	60·00	1·51	56·92	1·36
4	55·22	1·40	57·35	1·34	56·76	1·27
5	59·81	1·40	59·68	1·22	58·96	1·14
6	56·22	1·47	57·71	1·75	55·76	1·31
Overall Mean	59·91[a]*	0·55	59·26[ab]	0·57	58·58[b]	0·53

* Means with the same superscript are not statistically significantly different ($P < 0{\cdot}05$).

The final concentrations of the antibiotics in the plates were ampicillin, 50 μg/ml; streptomycin, 5 and 25 μg/ml; tetracycline, 20 μg/ml; chloramphenicol, 10 μg/ml; sulphathiazole, 550 μg/ml; trimethoprim, 50 μg/ml; kanamycin, 10 μg/ml; naladixic acid, 500 μg/ml; gentamicin, 2·5 μg/ml and spectinomycin 50 μg/ml.

RESULTS

The isolation rate of salmonellas from caecal contents and carcass surfaces during the trial shown in Table 1.

It was noted that caecal contents had a higher water content and lower volume in the pigs kept in lairage for 42 and 66 h.

Combined results from the two abattoirs showed caecal salmonella were isolated from 18·5 % of pigs killed on the first day, 24·1 % on the second and 47·7 % on the third day. There were differences in the caecal salmonella isolation rate between the two abattoirs with greater numbers of caecal salmonella at Abattoir A.

The isolation rate of salmonellas from carcasses at the 2 abattoirs showed 9·3 % of carcasses contaminated on day 1, 12·8 % on day 2 and 27·3 % on day 3. The majority of carcasses in the last group were detected on 2 of the 6 samplings.

Details of the most frequently isolated salmonella serotypes at each abattoir are given in Table 2. Thirteen salmonella serotypes were isolated from caecal contents and carcasses over the 6 weeks of the trial.

Significant differences were demonstrated between the caecal salmonella isolation rates on day 1 and day 3 in 2, 3 and 4.

Carcass weights are shown in Table 3. There was no significant difference in the weights over the 3-day period for each experiment but when all results were combined there was a significant drop in weight in pigs held in lairage for 3 days.

DISCUSSION

The only reported studies which have followed pigs from the same source

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through varying periods of lairage, using culture of caecal contents as the method of salmonella detection are those of Craven & Hurst (1982) and Schulz (1983).

Williams & Newell (1968) and Shotts Martin & Galton (1962) used rectal swabs to determine the presence of salmonellas in pigs on arrival at the abattoir. There are, however, problems with this approach as rectal swabs are often inefficient at recovering salmonellas from pigs (Haddock, 1970).

In the longitudinal study reported here, it was demonstrated that the number of pigs carrying salmonellas in the caeca increased significantly in pigs held for extended periods of time in lairage. In addition, the number of pigs carrying salmonellas in the caeca on the first day of lairage were low possibly because they were from a single source. This is in direct contrast to the pigs studied by Craven & Hurst (1982) which were from diverse sources and heavily infected after one day in lairage. A study by Gallwey & Tarrant (1979) suggests that mixing of pigs from several sources is stressful. This could in turn lead to increased salmonella transmission between pigs (Williams & Newell, 1968).

The results obtained in this study, although at variance with those of Craven & Hurst (1982) can be explained by the initial levels of salmonella carriage in the slaughter pigs studied. In the work of Craven & Hurst (192) the pigs were accumulated from several farms on a truck run and had probably reached a maximal level of caecal infection due to the handling and transport stress prior to their arrival at the abattoir. The social stress of mixing different groups of pigs probably would have been enough to ensure maximum spread of salmonellas between pigs. Williams & Newell (1968) observed that pigs transported to slaughter voided faeces and thus increased opportunities for transmission of salmonellas between pigs. In the study reported here, the pigs were probably less stressed as they were derived from the same unit and transported direct to the abattoir. Subsequently the stress of lairage, social mixing and the efflux of time allowed salmonellas to spread between pigs.

Our observations suggest that pen size and hygiene influence the build-up of salmonellas in pigs during lairage. The number of pigs held for 24 h in lairage with salmonellas in their caeca were significantly different between the two abattoirs. Pigs held in lairage at Abattoir A continued to have a higher prevalence of salmonellas in caecal contents, whereas the build-up of salmonellas in pigs at Abattoir F was slow. The pens at Abattoir F were smaller and the hygience was much better than at Abattoir A. Gallwey & Tarrant (1979) observed that pigs were less stressed when held in small lairage pens and were handled quietly.

The time spent in lairage could be used to manipulate the prevalence of salmonellas in pigs. If pigs come into lairage with a high prevalence of salmonella it may be possible to keep them in lairage until the number of infected animals is reduced. On the other hand, pigs with a low prevalence of salmonella should be slaughtered as soon as possible to avoid a build up of salmonella infection.

In order to reduce further the number of salmonellas entering the abattoir attention needs to be given to other features of lairage design and transport systems. Our observations suggest that build up of salmonella numbers will be minimized if pigs are transported directly to the abattoirs, handled quietly at all times and not mixed with animals from other sources.

In general, the same salmonella serotypes persisted over the 3-day lairage period

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for each experiment, suggesting that a relatively small number of salmonella types were initially present in the pigs. Basically the same salmonella serotypes were recovered from both the caecal contents and the carcasses in any one experiment. A high salmonella recovery rate for carcasses was generally associated with a high isolation rate from caeca. There were exceptions to this finding such as on day 1, in experiment 4, when the carcass contamination rate was high but only three pigs caecums yielded salmonella. In this case, equipment and operators may have been contaminated by an earlier batch of pigs. Thus, in our study, the source of carcass contamination was primarily intestinal salmonella infections and the extent of carcass contamination was determined by the number of salmonella entering the abattoir in the intestine of slaughtered pigs. The most important strategy in reducing salmonella contamination in pig slaughtering is therefore to manipulate pre-slaughter handling to minimize the multiplication of salmonella in pigs.

The results in Table 2 suggest that salmonella serotypes may differ in their ability to colonize pig skin. Some types such as *Salmonella anatum*, *S. agona* LD-, and *S. havana* appear to colonize more readily than *S. derby* and *S. give* (experiment 3) despite the fact that there were large numbers present in the caeca of pigs. The *S. give* isolated from pigs in experiment 4 was possibly a different strain.

All groups of pigs showed some fall in carcass weight. Usually, carcass weights were, on average, 2 kg less after 66 h in lairage when compared to carcasses from pigs kept only 18 h in lairage. Because of the variation in weights this difference was not statistically significant at all visits. Combining all results showed that there was a significant drop in weight between Day 1 and Day 3 in lairage. Thus increased time in lairage as well as increasing salmonella contamination of caecal contents and carcasses also results in carcass weight loss, in spite of feeding.

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