

## **Salmonella in the intestinal tract and associated lymph nodes of sheep and cattle**

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### SUMMARY

The distribution of salmonellas along the gastrointestinal tract and in associated lymph nodes were studied in 100 sheep and 100 cattle at slaughter. Animals were chosen from those slaughtered on the first day of the week, since this meant that they were likely to have been held at the abattoir for several days and thus to be at high risk of salmonella infection. The contents of the rumen, abomasum, ileum, caecum and rectum were sampled, together with the lymph nodes draining each of these sites.

Of the cattle, 77 were carrying salmonellas, including 61 with infected lymph nodes, whereas only 43 sheep were infected, 14 of them with infections in the nodes. The lower prevalence in sheep than in cattle might be explained by a shorter time between leaving the property and slaughter. In both species, within the gastrointestinal tract salmonellas were most frequently found in the caecum and rectum and least frequently in the abomasum. In cattle salmonellas were frequently present, usually in large numbers, in the lymph nodes draining the ileum, caecum and colon, but rarely in the ruminal and abomasal nodes; however this difference was not apparent in sheep. Over 70% of infected animals yielded more than one serotype, the maximum number isolated from any one animal being ten.

### INTRODUCTION

A number of workers in several countries have studied the incidence of salmonella infection in normal cattle and sheep at slaughter. Findings in countries other than Australia have ranged from no infected animals out of 300 sheep and cattle (Smith, 1959) to 8.1% infected out of 2755 sheep and cattle (Nottingham & Urselmann, 1961). In Australia, Daleel & Frost (1967) who examined several sites along the gastrointestinal tract of 2000 cattle found an incidence of 11.6%, while Grau & Brownlie (1965) found a rate of carriage in the rumen of 45% in 193 cattle. Studies have been made of the incidence of contamination by salmonellas in frozen boneless meat imported into Britain. During the 1960's the incidence in meat imported from Australia was 6% for beef and 9% for mutton, while from the Argentine it was 22% for beef and 39% for mutton, and in New Zealand mutton it was 8% (Hobbs, 1965; Hobbs & Gilbert, 1970); in beef imported from Holland the incidence was 7% (Dixon & Peacock, 1965). In a large, co-operative study in

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Australia from 1965 to 1973 (Paxton, 1974), 21031 samples of boneless beef and mutton from export meatworks throughout the country were examined for the presence of salmonellas and the rate of contamination was found to be 2% in beef and 5% in mutton. More recently we have shown that, in adult cattle which are slaughtered after being held for several days at the abattoir, the incidence of infection with salmonella is very high not only in the rumen (76%) but also in the mesenteric lymph nodes (54%); moreover, in many of the nodes concentrations of salmonellas are sufficiently high for them to be recovered by direct plating (Moo *et al.* 1980; Samuel *et al.* 1980*a*). A more detailed study of some of these infected cattle has suggested that, in the majority of clinically normal cattle which harbour salmonellas, the organisms are restricted to the gastrointestinal tract and its associated lymph nodes (Samuel *et al.* 1980*b*). The sites along the gastrointestinal tract which have been most frequently sampled in previous surveys have been the mesenteric lymph nodes (usually only those draining the small intestine: the jejunal nodes), and the contents of the distal colon or rectum; some workers have also examined the contents of the rumen, ileum or caecum, or have pooled the colonic and caecal lymph nodes with the jejunal nodes. There does not appear to have been any study in ruminants in which a number of sites along the gastrointestinal tract has been examined simultaneously together with the lymph nodes draining each part. Yet when a large proportion of slaughtered cattle is carrying salmonellas, often in high numbers, it is important to know this distribution not only as an aid to understanding the pathogenesis of the infection but also in order to institute measures to minimize the contamination of meat and edible offal during slaughter and inspection. Accordingly, a study was undertaken to determine the prevalence of salmonellas at given sites along the gastrointestinal tract and in the lymph nodes associated with them, in animals which were considered to be at risk of salmonella infection. The study was carried out in sheep as well as in cattle, since little was known about the prevalence of infection in this species in Australia and because sheep can be more conveniently examined and used as experimental animals than cattle.

#### MATERIALS AND METHODS

Sampling was carried out at the same abattoir as were our earlier studies (Samuel *et al.* 1980*a, b*), a Brisbane export abattoir which processed up to 800 head of cattle, and 3000 head of sheep, per day. One hundred cattle and 100 sheep were sampled, in groups of 4–6. In order to obtain as high an incidence of salmonella infection as possible, the animals were selected at random from among those slaughtered on the first working day of the week, as these were likely to have been held at the abattoir for several days. The cattle were all mature animals whereas the sheep were classed as 'lambs'; i.e. no permanent incisors had erupted. Samples from cattle were collected between June 1979 and January 1980, while sheep were sampled from April to June and from September to December, 1979. From each animal five samples of gut contents were taken from rumen, abomasum, ileum, caecum and rectum, and five sets of lymph nodes: 2–5 ruminal nodes, 1–2 abomasal, 3–5 jejunal, the caecal, and 1–3 colonic nodes. All samples were held at ambient temperature for up to 3 h before examination.

The lymph nodes were cut from the surrounding fat, using sterile instruments, and held in boiling water for 4 s in order to kill surface contaminants. They were then placed in plastic bags and weighed, and for every gram of tissue 5 ml of sterile distilled water was added. The nodes were then broken up in a Colworth 400 Stomacher for 2½ min, and 1 ml of the resultant mixture was inoculated into 10 ml each of tetrathionate broth (Oxoid CM29) and mannitol selenite broth (Oxoid CM399). Similarly 1 ml of each sample of gut contents was inoculated into 10 ml of each of the two broths. The broths were incubated for 24 h at 37 °C and each was plated onto bismuth sulphite agar (Gibco Diagnostics MO5600) and brilliant green agar (Oxoid CM329). After 24 h incubation at 37 °C, plates were examined and up to three suspect colonies were picked from each. These were tested in lysine decarboxylase broth (DIFCO 0215-02), purified on cystine lactose electrolyte deficient agar (CLED agar, Gibco Diagnostics M12 400), tested for agglutination against polyvalent O serum, Groups A to G (Wellcome Salmonella Agglutinating Serum) and finally tested in  $\sigma$ -nitrophenyl- $\beta$ -D-galactopyranoside broth (Wilson, Padron & Dockstader, 1971). All strains thus identified as salmonella were sent for serotyping to the Salmonella Reference Laboratory in Adelaide.

Samples were also inoculated directly onto selective media. From the mixture of lymph nodes in water, 0.2 ml was spread onto a 90 mm plate of MacConkey agar (Oxoid CM115), while the gut contents were diluted 1 in 100 in sterile distilled water and 0.2 ml of each was spread onto a plate of bismuth sulphite agar. These plates were incubated for 24 h at 37 °C, after which suspect colonies were counted and three colonies from each were tested and identified as described above.

## RESULTS

Salmonellas were detected in 77 out of the 100 cattle and in 43 of the 100 sheep. Of the cattle, 72 yielded salmonellas from the gut contents and 61 from the lymph nodes. In 42 of the 43 infected sheep salmonellas were found in the gut contents while in only 14 were they isolated from the lymph nodes. The frequencies of isolation from the various sites are shown in Table 1; figures in the table represent percentages, since in a small number of cattle one or more of the samples could not be obtained. Within the gastrointestinal tracts of both sheep and cattle the sites which most frequently yielded salmonellas were the caecum and rectum; more than 30 % of the sheep and more than 50 % of the cattle carried salmonellas in at least one of these sites. In both species salmonellas were least frequently found in the abomasum (16–17 %). Isolations of salmonellas by direct plating were made from only a small proportion of infected contents; in cattle they were obtained from all sites except the abomasum, and in sheep only from the intestinal sites. Where the concentrations of salmonellas could be estimated from the plate counts, they ranged in most animals from 500 organisms per ml, which was the lowest that could be detected, to 10000 per ml; however in one of the cattle the contents of ileum, caecum and rectum each yielded at least 100000 organisms per ml.

In cattle, there was a high incidence of salmonella infection in the lymph nodes which drained the intestines; over 50 % harboured salmonellas in the jejunal and caecal nodes and 39 % in the colonic nodes. Infections of the ruminal and abomasal nodes were rare. In sheep, on the other hand, there was a much lower incidence

Table 1. *Salmonella* isolations from different sites along the gastrointestinal tract and from the associated lymph nodes of 100 cattle and 100 sheep

	Percentage of animals positive for salmonella			
	Cattle		Sheep	
	By enrichment	By direct plating	By enrichment	By direct plating
Lymph nodes				
Ruminal	2	2	5	4
Abomasal	4	4	7	6
Jejunal	52	36	7	7
Caecal	53	38	10	7
Colonic	39	30	7	6
Contents				
Rumen	45	5	21	0
Abomasum	16	0	17	0
Ileum	27	4	30	4
Caecum	51	3	33	3
Rectum	57	6	35	1
Total positive in nodes	61	46	14	12
Total positive in contents	72	10	42	5
Total positive altogether	77	—	43	—

of infection in all the lymph nodes examined, but there was little difference between the nodes draining the intestines and those which drained the gastric complex. In both sheep and cattle, a high proportion of the infected lymph nodes yielded salmonellas by direct plating, indicating a concentration of at least 25 organisms per gram of tissue. In 38 cattle, and 10 sheep, at least one lymph node was estimated as carrying more than 100 salmonellas per gram. In addition out of 185 infected lymph nodes or set of nodes, 47 (25%) carried more than 1000 salmonellas per gram, these intense infections being evenly distributed among the jejunal, caecal and colonic nodes.

A total of 33 serotypes was isolated, 31 from cattle and 18 from sheep. These are shown in Table 2. In cattle, *S. anatum* was the most frequently isolated serotype, being obtained from 45 animals. In sheep, *S. havana* was the most common serotype, while in both species the next most frequently isolated serotype was *S. typhimurium*. Multiple serotypes were found in the majority of infected animals: in 60 cattle and 27 sheep. From one of the cattle, 10 serotypes were isolated, six from the gut contents and eight from the lymph nodes; four of the latter were isolated by direct plating. A further two cattle each yielded eight serotypes, one yielded seven, and six yielded six; of the sheep, two carried six serotypes, all of them present in the gut contents.

## DISCUSSION

The finding of a high incidence of infection in cattle, with multiple serotypes and large numbers of organisms in mesenteric lymph nodes, confirmed earlier findings in similar groups of cattle at the same abattoir (Moo *et al.* 1980; Samuel

Table 2. *Salmonella* serotypes isolated from lymph nodes and gut contents of 100 cattle and 100 sheep

Serotype	Cattle			Sheep		
	From nodes	From contents	Total	From nodes	From contents	Total
aberdeen	1	—	1	—	—	—
abony	1	1	2	—	—	—
adelaide	10	12	18	2	5	5
agona	1	2	3	—	—	—
anatum	31	34	45	1	5	5
bredeney	7	12	17	3	10	11
bukavu	1	—	1	—	—	—
chester	15	13	20	1	3	4
derby	—	1	1	1	4	4
eastbourne	2	1	2	—	—	—
give	10	11	15	1	4	4
havana	6	10	12	5	19	19
hvittingfoss	2	1	3	—	—	—
infantis	—	1	1	—	3	3
kottbus	2	1	2	2	9	10
litchfield	3	3	4	—	—	—
meleagridis	2	4	4	—	—	—
muenchen	9	12	17	—	2	2
new brunswick	1	1	2	—	—	—
newington	—	1	1	—	—	—
newport	—	—	—	1	3	3
onderstepoort	—	—	—	1	2	3
oranienburg	9	8	13	1	1	2
orion	1	2	3	—	2	2
potsdam	1	1	2	3	5	5
rubislaw	1	—	1	—	—	—
saint paul	8	9	12	—	—	—
san diego	1	—	1	—	—	—
senftenberg	3	4	4	—	2	2
singapore	1	1	2	—	—	—
tennessee	—	1	1	2	7	7
typhimurium	25	23	32	5	10	12
virchow	1	1	2	—	—	—

*et al.* 1980a, b). The incidence of infection in sheep, although significantly lower than in cattle, was much higher than that reported in overseas studies. In a survey in England, Smith (1959) failed to find salmonellas in the mesenteric lymph nodes of 100 sheep while, in India, Kumar, Saxena & Gupta (1973) examined 812 sheep and found that 17 (2.1 %) carried salmonellas in the rectal contents and 10 (1.2 %) in the lymph nodes draining the small and large intestines. In New Zealand, slightly higher rates of infection have been found. Nottingham & Urselmann (1961) found three out of 33 sheep to be positive for salmonella in the mesenteric lymph nodes. Kane (1979) examined 2027 sheep and found that, although the overall incidence of infection was 1.7 % in the caecal contents and 4.0 % in the pooled mesenteric nodes, variations with ages and seasons meant that the incidence was much higher in certain groups: for example 32 % in 2- and 4-tooth sheep examined during the month of April. It must be stressed however that the sheep which were

sampled in the present study were from groups which were suspected of being at risk of salmonella infection, in that they were slaughtered after a weekend. The most striking difference between the cattle and sheep was in the proportion of infected animals which harboured salmonellas in the lymph nodes. Of the cattle which were positive, 79% were found to have infected lymph nodes, whereas the corresponding figure for the sheep was 31%. This need not necessarily be taken to indicate a difference between the two species in their resistance to invasion by salmonellas; it is more likely to reflect differences in management of the animals before slaughter, and possibly the difference in ages. As has already been pointed out elsewhere (Samuel *et al.* 1980*a*), the high incidence of salmonella infection found in cattle in Australia, in contrast to other countries, is probably due to the long distance over which animals must be transported for slaughter which provides opportunities for the spread of infection between animals and for the multiplication of organisms in animals which are fed only at long intervals. The high incidence found in sheep in this study may be similarly explained. However, whereas the cattle sampled had been held at the abattoir for at least 4, and often 6, days before slaughter and had usually been obtained from sale-yards, most of the sheep had arrived at the abattoir 3 days or less before slaughter, and many had come directly from the farm of origin. There would thus be less time for infections to develop after leaving the farm and, in sheep which did not pass through saleyards, less exposure to new infections. This may help to explain the difference between cattle and sheep in the incidence of both carriage of salmonella in the gut and salmonella infection in the lymph nodes. In some groups of sheep the frequency of carriage of salmonellas was very high, with a large proportion having infected lymph nodes. In a separate study at the same abattoir, 20 lambs and 20 mature sheep were sampled on one day, only the rectal contents and the caecal lymph nodes being examined; 30 (75%) of these yielded salmonellas from the rectal contents and 17 (43%) from the lymph node, there being no difference in frequency between sheep and lambs (Samuel, 1979 unpublished data).

The multiple serotypes which were found in the majority of infected animals suggested that many of the infections resulted from numerous exposures to salmonellas from different sources, possibly over periods of several days. This made it difficult to draw conclusions relating to the pathogenesis of the infections. However, there is no evidence that the pathogenesis in ruminants differs greatly from that in monogastric animals. The distribution of salmonellas in the lymph nodes of the cattle did support our earlier suggestion that the organism invaded the host from the intestines rather than from the gastric complex. Carter & Collins (1974), who investigated the pathogenesis of salmonellosis in mice, showed that the primary sites of invasion were the distal ileum and possibly the caecum. Within a few hours of inoculation over 99% of the inoculum was destroyed, but some of the surviving salmonellas passed to the Peyer's patches in these regions. From there they passed to the mesenteric lymph nodes and thence by 48 h post-infection to the liver and spleen, where multiplication led to a secondary bacteraemia and death within 10 days. In ruminants, the sequence of events following inoculation has been less thoroughly investigated. In a monogastric animal, ingested salmonellas have no opportunity to multiply before reaching the intestine, whereas in a ruminant the forestomachs may provide an environment where salmonellas can grow. Grau,

Brownlie & Roberts (1968) have shown that growth may occur in the rumen of cattle which are fed only at long intervals; they found that salmonellas could multiply by as much as  $10^5$ . Even though these organisms may not invade the host directly from the rumen, the presence of even moderate concentrations of salmonellas in such a large volume is important both as a source of contamination after slaughter and as a source of infection for the rest of the gastro-intestinal tract. However, it is probable that a large proportion of the salmonellas from the forestomachs would be killed in the acid environment of the abomasum, and in support of this it should be noted that a lower frequency of salmonella carriage was found in the abomasum than in any of the other sites in the gastro-intestinal tract.

The present study is perhaps the first in which samples have been examined simultaneously from a series of sites along the intestinal tract and associated lymph nodes, and in which direct plating techniques have been used to provide information on the numbers of organisms present: a matter of considerable significance. It is important to interpret our findings of a high incidence of salmonella infection in various parts of the intestinal tract and related sites with some caution, since for the purposes of our study animals were selected which were likely to have a high incidence of infection; moreover, the techniques used were probably more sensitive than some of those employed in earlier studies. The degree of contamination of meat with salmonellas depends on both the incidence in the animals before slaughter and the standards of slaughter hygiene at the abattoir. As indicated earlier, there may be a high incidence of salmonella in the intestinal tract under some circumstances, but the large survey reported by Paxton (1974) revealed an incidence in Australian boneless meat of only 2% for beef and 5% for mutton.

The control of salmonellosis in most countries presents an almost insuperable problem at the present time. It is well to recall, however, that the control of bovine tuberculosis seemed an equally daunting task in the first decades of this century. While the pasteurization of milk reduced the danger to man not only of bovine tuberculosis but also of other milk-borne diseases, eradication of bovine tuberculosis was effected finally in the more advanced countries by the slaughter of infected animals detected by the tuberculin test (Francis, 1958). It is unlikely however, that the eradication of human salmonellosis of the food poisoning type will be effected by a slaughter policy of infected animals as most human food poisoning results from the mishandling during preparation and cooking and storage before consumption of the flesh of healthy animals and fowl contaminated at slaughter with salmonellas present in the gut contents. A reduction in the holding time of animals at abattoirs and improvement in abattoir hygiene and slaughter techniques would appear to be the most effective methods of control now available. Various other efforts are being made to reduce the overall incidence of salmonella infection in animals, especially in the Scandinavian countries. Pasteurization has been effectively applied to eliminate salmonellas from bulk egg products and an experimental technique for the 'pasteurization' of carcasses of sheep and, potentially, of other species has been reported (Smith & Graham, 1978).

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