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A study of the susceptibility of cattle to oral infection by salmonellas contained in raw sewage sludge

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SUMMARY

Raw sewage sludge, containing up to 10^5 naturally occurring salmonellas/l, was included in the diet of one group of cattle at the rate of 1 l/animal/day and in a second group at the rate of 1 l/animal/week. Sterilized sludge, to which had been added 10^5 *S. dublin*/litre, was included in the diet of a third group of animals at the rate of 1 l/animal/day.

Salmonellas were isolated from all samples of raw sewage sludge but were not isolated from the faeces or carcasses of animals fed on the sludge. Salmonellas were isolated from the faeces of one animal and the carcasses of two animals fed on sterilized sludge to which *S. dublin* had been added.

INTRODUCTION

Water authorities in Britain dispose of sewage sludge, either after digestion or raw, on grazing land. They would wish to increase the amount disposed of in this way. The value of sludge to farmers lies in its water, humus and nutrient content, but it also contains bacteria which are potentially pathogenic to grazing animals (Wray, 1975). The bacteria most frequently found in sewage sludge, which could infect grazing animals, are salmonellas.

The aim of the present experiment was to obtain an estimate of the susceptibility of cattle to oral infection by salmonellas contained in raw sewage sludge.

MATERIALS AND METHODS

Animals

Twelve Friesian heifers aged 10–12 months, which had been born and reared on the Institute's farms, were used. They had been housed during the winter and at the start of the experiment would normally have been put out to graze. The animals were divided, at random, into three groups of four animals and the groups were housed separately in loose boxes.

Raw sludge

Samples of raw sludge were collected twice weekly from a local sewage works and stored at 4 °C until required. Each sample was completely used within 4 days

of collection. A total of eight samples were collected and an estimate of the salmonella content of each sample was obtained, just before feeding, by the 'Most Probable Number' technique (Taras, Greenberg, Hoak & Rand, 1971). Samples of sludge were inoculated into selenite brilliant green broth (Difco) (SBG) and incubated at 43 °C. After 24 and 48 h incubation all broths were inoculated on modified brilliant green agar (Oxoid). Plates were incubated at 37 °C and examined after 24 and 48 h. Non-lactose and non-sucrose fermenting bacteria, resembling salmonellas in colony morphology, were identified serologically according to the method of Kauffmann (1972). Sludge was sterilized by heating in an autoclave for 30 min at a pressure of 20 lb/in^2.

Salmonella dublin

An aerogenic smooth strain of *S. dublin* (3246) isolated from a case of abortion in a dairy cow was used. One ml of a 1/10000 dilution of a bactotryptose broth culture, containing approximately 10^5 *S. dublin*/ml, was added to each litre of sterilized sludge. The methods of producing and counting bacterial suspensions have been described (Hall & Jones, 1977).

Diet

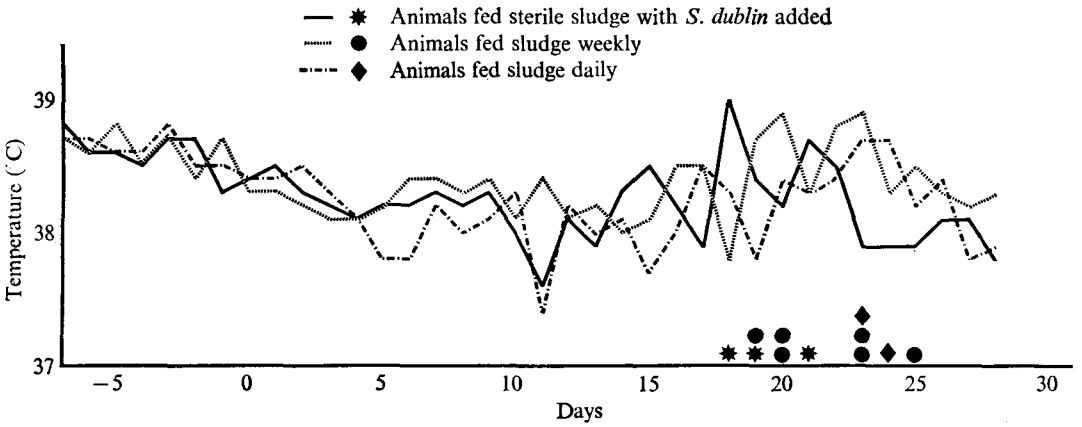
One week before the start of the experiment the heifers were introduced to a silage diet. From the start of the experiment, which lasted 28 days, one group of four animals was fed, daily, silage to which 4 l of raw sewage sludge had been added. The second group received the same diet on 1 day in each week and received uncontaminated silage on the other 6 days. The third group received daily silage containing 4 l of sterilized sewage sludge containing 10^5 *S. dublin*/l. When the experimental ration had been consumed animals were fed barley straw. Water was available *ad libitum*.

Observations

During the experiment and for 1 week before its commencement rectal temperatures were taken each morning before feeding, and the animals examined for signs of illness. Samples of faeces were then removed from the rectum and approximately 1 g portions of faeces from each animal were enriched in Rappaport and SBG broth and salmonellas isolated and identified as described above.

Post-mortem examinations

At the end of the experiment the animals were killed in the conventional manner using a humane captive bolt pistol followed by exsanguination. Carcasses were examined for the presence of grossly visible lesions and samples were removed for bacteriological examination which were: ruminal contents, ruminal wall, omasal contents, omasal wall, abomasal contents, abomasal wall, samples of contents, wall and associated mesenteric lymph node from four sites in the small intestine, caecal contents, caecal wall, caecal lymph node, colic contents, colic wall, colic lymph node, rectal contents, liver, lung, spleen, gall-bladder wall, bile, hepatic lymph node, bronchial lymph node and retropharyngeal lymph node.



Figl 1. Mean daily rectal temperatures for each group of animals. Each symbol represents one animal with a significantly raised body temperature on that day.

Ruminal, omasal, abomasal, small intestinal, caecal, colic and gall-bladder walls were washed twice to remove surface contamination. Surface contamination was removed from other tissues by dipping in absolute alcohol and igniting. Isolation of salmonellas by enrichment was made by placing 1 g portions of tissue in Rappaport broth and SBG broth, as previously described (Hall & Jones, 1977).

RESULTS

Salmonella content of sludge

The number of salmonellas isolated from each sludge sample is shown in Table 1. Fourteen *Salmonella* serotypes were isolated: *S. newhaw* (4 samples), *S. saintpaul* (3), *S. heidelberg* (4), *S. typhimurium* (3), *S. paratyphi B* (2), *S. oranienburg* (2), *S. kaapstad* (2), *S. senftenberg*, *S. panama*, *S. bournemouth*, *S. indiana*, *S. bredeney*, *S. infantis*, and *S. hadar*.

The mean number of *S. dublin* added to each litre of sterilized sludge/day was $1{\cdot}11 \pm 0{\cdot}17 \times 10^5$ (range $0{\cdot}80$–$1{\cdot}5 \times 10^5$).

Diet

Inclusion of raw sludge in the silage resulted in some reluctance to eat on the first 2 or 3 days of the experiment but once the animals had become accustomed to the inclusion of sludge the feed was rapidly eaten. There was no reluctance to eat silage containing sterilized sludge.

Rectal temperatures

Mean rectal temperatures were calculated each day for each group of animals and are shown in Fig. 1. Mean rectal temperatures were also calculated from each animal during the pretreatment period. During the treatment period an animal was considered to have a significantly raised body temperature when its rectal temperature was at least two standard deviations greater than the mean of the

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Table 1. *The number of salmonellas, and serotypes, isolated from each sludge sample*

Sample	Serotypes	Salmonellas per litre
1	*S. senftenberg, S. saintpaul*	$5{\cdot}3 \times 10^2$
2	*S. heidelberg, S. oranienburg, S. saintpaul S. typhimurium, S. paratyphi B*	$1{\cdot}1 \times 10^4$
3	*S. paratyphi B, S. typhimurium, S. newhaw*	$3{\cdot}4 \times 10^2$
4	*S. heidelberg, S. newhaw, S. kaapstad*	$1{\cdot}1 \times 10^5$
5	*S. heidelberg, S. typhimurium*	$2{\cdot}4 \times 10^3$
6	*S. panama, S. newhaw, S. kaapstad, S. bournemouth, S. indiana*	$4{\cdot}6 \times 10^2$
7	*S. bredeney, S. infantis, S. hadar*	$2{\cdot}4 \times 10^3$
8	*S. oranienburg, S. newhaw, S. heidelberg, S. saintpaul*	$2{\cdot}4 \times 10^3$

pretreatment period. Significantly raised rectal temperatures were detected on 11 occasions between the 18th and 25th days of the experiment (see Fig. 1).

Clinical observations

Coughing, accompanied by a muco-purulent oculo-nasal discharge, was observed in animals in all three groups between the 18th and 25th day of the experiment. Diarrhoea was not observed in any animal during the course of the experiment.

Isolation of salmonellas from faeces

Salmonellas were not isolated from any animal in the week before the start of the experiment nor were they isolated from any of the animals which received unsterilized sludge. *S. dublin* was isolated on the 13th and 14th days from the faeces of one animal (425) in the group which received *S. dublin* in sterilized sludge.

Post-mortem observations

Abnormalities were not detected in the group of animals which had received unsterile sludge daily. Collapsed and consolidated lobules were seen in the lungs of all the animals fed on unsterilized sludge weekly and in three out of four animals fed on sterilized sludge to which *S. dublin* had been added.

Isolation of salmonellas from tissues

Salmonellas were not isolated from the tissues of any of the animals which received unsterilized sludge. *S. dublin* was isolated from two animals (49, 437) in the group which received *S. dublin* in sterilized sludge. Isolations were made from the following tissues. Animal 437: omasal wall, abomasal wall, small intestinal wall (3rd site), small intestinal contents (4th site) colic lymph node and rectal contents. Animal 49: small intestinal contents (4th site) and rectal contents. Isolations were not made at post-mortem examination from the animal (425) which had excreted *S. dublin* in its faeces during the experiment.

DISCUSSION

Fourteen different serotypes of *Salmonella* were isolated from the sewage sludge samples and all are known to be associated with disease in domestic animals. The numbers in which they were isolated were extremely variable ranging from $3{\cdot}4 \times 10^2$/l to $1{\cdot}1 \times 10^5$/l, but are representative of the numbers in which such organisms may be present in sewage sludge (McCoy, 1957; Rennison & Jones, unpublished observations). The numbers present in any sample will depend upon the time at which the samples were taken and the level of excretion of salmonellas by the human and animal population from which the sewage is derived.

The records of rectal temperatures show a progressive fall in the mean temperature for each group from day -7 to $+4$. This is most probably the result of decreasing excitement in the animals as they became accustomed to having their rectal temperature measured. The records of rectal temperatures, when evaluated in conjunction with clinical observations and post-mortem findings, suggest that there was an outbreak of respiratory tract infection in all groups between days 18 and 25. This is unlikely to have been associated with sludge feeding.

Two levels of exposure to unsterile sludge were chosen to estimate the susceptibility of cattle to oral infection by salmonellas. The higher was selected to be greater than would be expected under normal farm conditions in which cattle graze pasture spread with sludge. Feeding sludge freshly added to silage every day exposed the cattle to many more salmonellas than a typical farming situation where a single application of sludge would be followed by reduction of salmonellas on grass (Taylor & Burrows, 1971). The group which received, daily, a litre of sterilized sludge, to which 10^5 *S. dublin* had been added, served as a positive control group. It was expected that this group which received sludge containing a concentration of salmonellas equal to the highest concentration found in natural sludge might become infected. A negative control group, fed uncontaminated silage, was not included since animals and fodder produced on the Institute were used and there is no history of salmonellosis in the cattle herds of the Institute. Bacteriological examinations made in the week before the start of the experiment shows that the animals were free from salmonellas.

The isolation, during the experiment, of *S. dublin* from the faeces of one animal in the group which received *S. dublin* in sterilized sludge and from samples taken at the end of the experiment from two other animals in the same group shows that the organism was established in the positive control group. Failure to isolate salmonellas from either of the two groups which received normal sludge suggests that they did not become infected. This is in agreement with the results of Crawford & Frank (1940) who fed sewage effluent to six cattle for 6 months without producing illness. They did not, however, examine samples of the effluent consumed for the presence of salmonellas. While it is not possible to conclude from the present experiment that salmonellas in sewage sludge do not infect cattle the results suggest that this is unlikely to occur except under extreme conditions of prolonged exposure to heavy contamination or when the susceptibility of the cattle might be unusually high (Aitken, Jones, Hall & Hughes, 1976). In Switzerland seasonal

increases in the incidence of isolation of salmonellas from adult cattle have been associated with the spreading of large quantities of sewage sludge (Hess & Breer, 1975) and in Holland regular disposal, to land, of sewage sludge on a dairy farm was thought to have raised the percentage of cows infected with salmonellas significantly above the percentage infected in the whole country (Strauch, 1977). The results of the present investigation show, however, that the risk of infection is unlikely to be measured by further laboratory experiments and it may be more appropriate to monitor the effects of the practice over a number of years under normal farming conditions.

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