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An investigation into the potential hazard to animal health of effluent sludge from dairy factories

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SUMMARY

Sixty-three samples of the more solid material (sludge) separated from the effluent plants of dairy factories were examined for the presence of salmonellas and brucellas. Salmonellas were isolated from two samples (*S. heidelberg.* [1]; *S. indiana* [1]). No brucellas were isolated. None of the samples supported the growth of *S. dublin*. Salmonellas added to effluent sludge at a concentration of 10^6 organisms/ml. survived less than 70 days.

The sludge from dairy factory effluent plants does not appear to be a source for the spread of salmonellosis or brucellosis.

INTRODUCTION

Waste materials produced during the manufacture of dairy products are treated to reduce their pollutive capacity and the sludge fraction removed from effluent treatment plants is commonly spread on farm land.

Pathogenic bacteria such as salmonellas and brucellas may be excreted in the milk of infected animals or milk may be contaminated with small quantities of faeces. It has therefore been suggested that an analogy may exist between the health hazards caused by the application of slurry to pasture and the application of effluent sludge from dairy factory waste treatment plants. Jones & Matthews (1975) reported that slurry from 11 % of dairy and beef farms in England and Wales was infected with salmonellas and might constitute a health hazard to grazing animals when spread on pasture.

The purpose of the work described here was to assess the extent to which pathogens gain access to dairy wastes and whether they are present in numbers sufficient to constitute a hazard to grazing animals when the effluent sludge is spread on land.

MATERIALS AND METHODS

Dairy effluent sludge

Samples of effluent sludge from dairy factories were collected at sludge withdrawal points of the effluent treatment plants. The temperature of the sludge at

the time of sampling was recorded. The samples were placed in 100 ml. bottles and returned by first class mail to the Institute for Research on Animal Diseases. A total of 60 samples were received. Three samples of 500 ml. were also received and were used as described below.

Isolation and characterization of salmonellas

On arrival at the laboratory the samples were mixed and enriched in Difco selenite brilliant green enrichment broth (SBG), Rappaport broth (Rappaport, Konforti & Navon, 1956) and brilliant green MacConkey broth (Smith, 1959). Ten ml. of each sample was placed into 2 replicate 100 ml. amounts of each of the enrichment broths. The Rappaport broths were incubated at 37° C., and the SBG at 43° C. One brilliant green MacConkey broth was incubated at 37° C. and the other at 43° C. After 24 and 48 hr. incubation all were inoculated on modified brilliant green agar (Oxoid CM329) with the addition of sulphadiazine (BDH) (120 mg./l.). Plates were incubated at 37° C. and examined after 24 and 48 hr.

Non-lactose and sucrose fermenting bacteria resembling salmonellas in colony morphology were identified biochemically according to the method of Edwards & Ewing (1968) and serologically according to the method of Kauffman (1972).

Examination for brucellas

Samples of sludge were mixed and streaked heavily on three replicates of albimi agar (Joint FAO/WHO, Expert Committee on Brucellosis, 1958) and incubated for 72 hr in an atmosphere of 10% carbon dioxide. Colonies resembling those of *Brucella abortus* morphologically were examined for agglutination in mono-specific *Br. abortus* antiserum.

Total colony count and coliform count

Appropriate dilutions of effluent sludge in saline were incubated aerobically on nutrient agar (Oxoid CM3) at 20° C. for 72 hr. and counted by the method of Miles & Misra (1938). The coliform count was determined by the 'surface viable count by spreading method' of Cruickshank (1968). Volumes of 0·1 ml. of appropriate dilutions of effluent sludge in physiological saline were spread over the surface of MacConkey agar plates and incubated at 37° C. for 24 hr.

pH and total solids concentration

pH of the undiluted sample was measured with a membrane electrode. Ten grams of each sample was heated in a hot air oven at 104° C. for 24 hr. The residual solids were weighed and recorded as a percentage of the original weight of the wet sample.

Growth supporting capacity of samples

Ten ml. of each sample was seeded with *S. dublin* from an 18 hr. bacto-tryptose broth culture to a concentration of 10^5 *S. dublin*/ml. The seeded sample was incubated at 20° C. for 72 hr. and then counted by the method of Miles & Misra (1938) on modified brilliant green agar (as above).

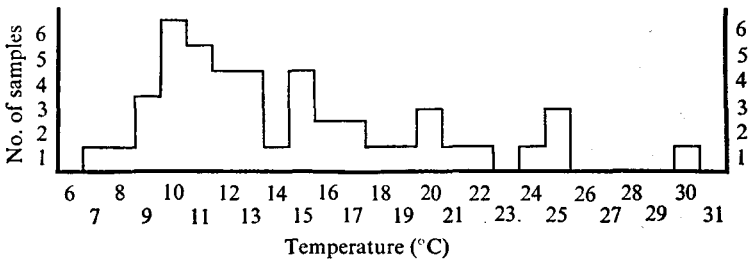

Fig. 1. The temperature of samples measured on collection at the effluent plants.

The survival of S. dublin *and* S. typhimurium *in dairy effluent*

The three 500 ml. samples described above were used to investigate the survival of *S. dublin* and *S. typhimurium* in stored effluent. Each sample was used in 30 ml. volumes and replicates of each sample were seeded with either *S. dublin* or *S. typhimurium* to a concentration of approximately 10^5 salmonellas/ml. and incubated at 10° C., 20° C. or 30° C. according to the scheme shown in Tables 1 and 2.

Each sample was examined for extraneous salmonellas before seeding by the method previously described. Each 30 ml. volume was sampled after 18 hr., 2 days, 6 days and then at weekly intervals until no further salmonellas could be isolated or until 70 days when the remaining samples were discarded. The pH was determined at the time of each sampling and the number of salmonellas present was determined by the method of Miles & Misra (1938) on modified brilliant green agar (as above.) When the number of salmonellas/ml. in a sample fell to a figure at which counting was difficult a simple present or absent result was obtained by enrichment of 1 ml. volumes of samples in 9 ml. volumes of SBG and Rappaport broth (as above).

RESULTS

Temperature of effluent at time of sampling at the effluent treatment plant

The temperature of samples at the time of sampling is shown in Fig. 1. This ranged from 7° C. to 30° C.

Isolation and characterization of salmonellas

Two strains of *Salmonella* were isolated from two separate samples. These were identified as *S. heidelberg* and *S. indiana*. *S. heidelberg* was isolated from both SBG broths and from one Rappaport broth, while *S. indiana* was isolated from 1 SBG broth.

Examination for brucellas

No brucellas were isolated.

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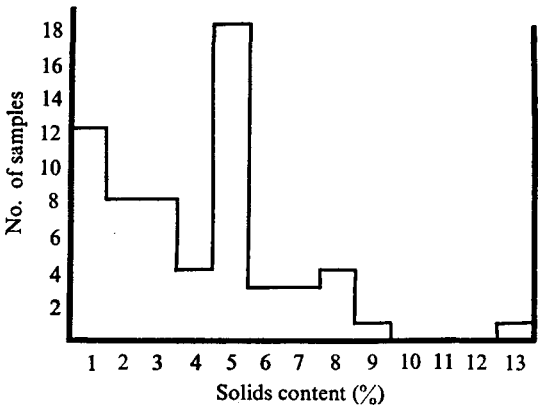

Fig. 2. The solids content of 63 samples of dairy factory effluent sludge. One sample not included had a solids content of 51·1 %.

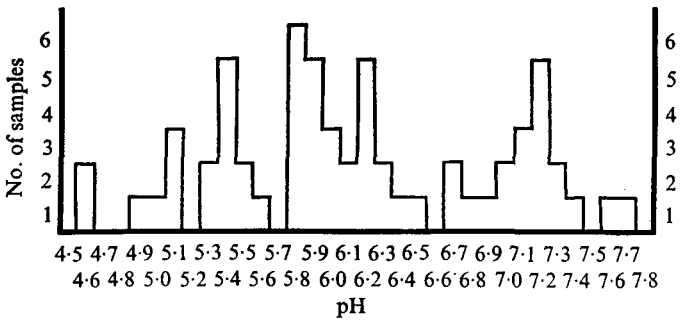

Fig. 3. The pH of 63 samples of dairy factory effluent sludge. Two samples not included had pH's of 11·8 and 12·3.

Total colony count and coliform count

The total colony count ranged from $< 10^2$ organisms/g. to 10^9 organisms /g. The coliform count ranged between < 10/g. and > 10^7/g. Most samples (70 %) contained between 10^5 and 10^7 coliforms/g.

Total solids content and pH

The total solids content of the samples is shown in Fig. 2. This ranged between less than 1 % (12 samples) to 12·8 %. There was one sample outside the range at 51·1 %.

The pH of the samples is shown in Fig. 3. For most this ranged from 4·6 to 7·7 but there were two samples outside the range at 11·8 and 12·3.

Sixty samples were seeded with *S. dublin* to a concentration of 10^5/ml. After 72 hr. the number of salmonellas present had declined in 57 samples. In three samples the number of *S. dublin* had remained constant or risen slightly to $1{\cdot}1 \times 10^5$/ml., $1{\cdot}8 \times 10^5$/ml., and $2{\cdot}2 \times 10^5$/ml., respectively.

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Table 1. *The survival of* S. dublin *in three samples of stored dairy effluent sludge*

Sample	Storage temperature (° C.)	Concentration of *S. dublin*/ml. after 18 hr.	2 days	6 days	14 days	22 days	27 days	34 days	43 days	57 days	70 days
A	10	1×10^4	3×10^2	7×10^1	−	−	−	.	.	.	.
	20	+	+	−	−	−	.	.	.	.	.
	30	−	−	−	.	.	.	.	.	.	.
B	10	+	+	+	+	+	+	+	+	+	+
	20	1×10^4	+	+	+	+	+	+	−	−	−
	30	4×10^4	+	+	+	−	−	−	.	.	.
C	10	1×10^5	1×10^5	5×10^4	+	+	+	+	+	+	+
	20	1×10^5	8×10^4	3×10^4	+	+	+	+	−	−	−
	30	4×10^4	3×10^4	3×10^1	−	−	−	.	.	.	.

The initial concentration of *S. dublin* was 1×10^5/ml.
+, *S. dublin* isolated by enrichment.
−, *S. dublin* not isolated by enrichment.

Table 2. *The survival of* S. typhimurium *in three samples of stored dairy effluent sludge*

Sample	Storage temperature (° C.)	Concentration of *S. typhimurium*/ml. after 18 hr.	2 days	6 days	14 days	22 days	27 days	34 days	43 days	57 days	70 days
A	10	2×10^3	3×10^1	−	−	−	.	.	.	.	.
	20	+	+	−	−	−	.	.	.	.	.
	30	−	−	−	.	.	.	.	.	.	.
B	10	6×10^4	3×10^4	3×10^3	3×10^3	5×10^2	4×10^2	3×10^2	+	+	+
	20	5×10^4	2×10^4	2×10^3	4×10^2	+	+	+	+	−	−
	30	7×10^4	1×10^4	5×10^2	+	−	−	−	.	.	.
C	10	7×10^4	1×10^4	+	+	+	+	+	+	+	+
	20	2×10^4	3×10^2	+	+	+	+	−	+	−	+
	30	2×10^4	7×10^3	2×10^2	−	−	−	.	.	.	.

The initial concentration of *S. typhimurium* was 7×10^4/ml.
+, *S. typhimurium* isolated by enrichment.
−, *S. typhimurium* not isolated by enrichment.

The survival of S. dublin *and* S. typhimurium *in dairy effluent sludge*

The survival of *S. dublin* amd *S. typhimurium* in stored dairy effluent sludge is shown in Tables 1 and 2. There was a rapid fall in the numbers of seeded organisms in most samples and after 14 days storage the numbers had fallen to insignificant levels in all but 2 samples. After 70 days salmonellas could still be recovered from 5 samples by enrichment. There was no significant difference in survival between the two serotypes of *Salmonella*. Both declined rapidly in sample A and survived for a considerable time (up to 70 days) in samples B and C. A low temperature of storage encouraged survival of both *S. dublin* and *S. typhimurium* in all three samples.

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DISCUSSION

The isolation of salmonellas from only 2 of 63 samples of effluent sludge is low considering the wide sources from which the dairy factories collect their raw materials (mainly milk) and indicates that salmonellas may be removed during treatment of the waste. Similar results were obtained by Koser (1954), who isolated *S. enteritidis* from 1 of 370 creamery wastes, Endres (1955) who isolated *S. enteritidis* from 1 of 58 samples and Meis (1965) who failed to isolate *Salmonella* from 515 samples taken from 7 dairies and a condensed milk factory. It is also interesting to note that the salmonellas isolated were probably present in small numbers.

The samples examined varied considerably in their physical composition (Figs. 2 and 3) and temperature (Fig. 1) and yet *S. dublin* seeded into effluent sludge stored at a representative temperature of 20° C. did not multiply nor did it survive in large numbers. Survival was, however, enhanced at 10° C. When spread on pasture the number of salmonellas present would be unlikely to constitute a hazard to grazing animals.

The presence of salmonellas in 2 samples does not indicate a public health hazard. Salmonellas are removed from milk and milk products by pasteurization. However, the samples of effluent sludge examined could be expected to have contained waste material which had not been subjected to the pasteurization process. The concentration of salmonellas in such material would have been reduced by dilution with pasteurized waste materials and further reduced during the waste treatment process.

Brucellas were not isolated from any of the samples and although the method used for the isolation of brucellas was not as sensitive as the enrichment for salmonellas there is no evidence that dairy effluent sludge could be responsible for the dissemination of brucellosis. Moreover, there is no suggestion from epidemiological studies that brucellosis is spread by this means.

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