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Examination of slurry from cattle for pathogenic bacteria

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SUMMARY

One hundred and eighty-seven samples of slurry from cattle were examined for the presence of salmonellas, pathogenic leptospires and brucellas. Small numbers of salmonellas, generally less than 1/g., were isolated from 20 samples (11 %). These were *S. dublin* (12), *S. typhimurium* (4), *S. indiana* (1), *S. bredeney* (1), *S. cerro* (1) and *S.* unnamed 4,12:d:—(1).

Leptospires were isolated from 56 samples (30 %) but none was pathogenic for hamsters. No brucellas were isolated. The results of this survey are discussed in relation to the epidemiology of salmonellosis.

INTRODUCTION

Since many pathogenic micro-organisms may be excreted in the faeces of infected animals slurry could be a potential hazard to farm animals when applied to pasture. Rankin & Taylor (1969) demonstrated that *S. dublin* can gain access to slurry systems and Jack & Hepper (1969) implicated slurry in an outbreak of *Salmonella typhimurium* infection in cattle. However, there is no information on how often slurry systems contain potentially dangerous pathogens, or on the levels of infection likely to be found.

The purpose of the work described here was to examine a number of systems and to assess the degree to which they were contaminated with pathogenic micro-organisms.

MATERIALS AND METHODS

Slurry samples

Slurry was obtained from farms chosen at random from the eight Agricultural Development and Advisory Service (ADAS) regions of England and Wales. ADAS officers collected 100 ml. samples of slurry from a point near to or at the inlet of the system. The samples were placed in sterile 100 ml. bottles and returned to the laboratory by first class mail. A total of 187 samples were received.

Examination of samples

On arrival each sample was mixed thoroughly and examined for the presence of salmonellas, brucellas and leptospires. Ten ml. was stored at $-20°$ C.

Isolation, enumeration and characterization of salmonellas

Each sample was enriched in Difco selenite brilliant green enrichment broth (SBG) and in Rappaport's broth (Rappaport, Konforti & Navon, 1956) as shown below:

1 g. of slurry in 9 ml. SBG – 3 replicates

1 g. of slurry in 9 ml. Rappaport's broth – 3 replicates

1 g. of slurry plus 9 ml. sterile physiological saline in 10 ml. of double strength SBG

2·5 g. of slurry plus 7·5 ml. sterile physiological saline in 10 ml. of double-strength SBG

5·0 g. of slurry plus 5·0 ml. sterile physiological saline in 10 ml. of double-strength SBG

10 g. of slurry plus 40 ml. of sterile physiological saline in 50 ml. of double-strength SBG

The Rappaport's broths were incubated at 37° C. and the SBG at 43 °C. After 24 and 48 h. incubation all broths 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 h.

Non-lactose and -sucrose fermenting bacteria resembling salmonellas in colony morphology were identified biochemically and serologically. Samples shown by enrichment to contain salmonellas were examined to determine the number of salmonellas present. The frozen sample was allowed to thaw at room temperature and 0·1 ml. volumes of appropriate dilutions were spread over the surface of modified brilliant green agar (with the addition of 120 mg./l. sulphadiazine) according to the 'surface viable count by spreading' method of Cruickshank (1968); the plates were incubated at 37° C. and colonies agglutinating in *Salmonella* polyvalent O serum (Wellcome Laboratories) were counted.

Salmonellas were identified biochemically according to the method of Edwards & Ewing (1962) and serologically according to the method of Kauffmann (1972). Serotypes other than *S. dublin* and *S. typhimurium* were identified by the Central Public Health Laboratory, Colindale.

Organisms identified as *S. dublin* were placed into biotypes according to the methods of Walton (1972) and Hall & Taylor (1970). Organisms identified as *S. typhimurium* were phage-typed by the Enteric Reference Laboratory, Colindale.

All strains isolated were tested for gas production (aerogenicity) in peptone water containing 1 % glucose (Walton & Lewis, 1971) and for agglutination in 1/500 neutral acriflavine solution.

Examination for leptospires

Twenty-five ml. of each sample was centrifuged at 800 *g* for 10 min. and the supernatant fluid removed. The moist deposit was mixed and spread over the surface of a sterile 0·22 μm. membrane disk (Millipore), placed on the solid medium of Stuart (1946) with the addition of fungizone (50 μg./ml.) and fluorouracil

(100 μg./ml.). The medium with the disk was incubated at 30° C. for 18 h., after which the disk was removed and incubation continued for up to 15 days.

Colonies morphologically resembling those of leptospires were placed in liquid Tween 80 albumin medium (Ellinghausen, 1965), and incubated at 30° C. for up to 10 days. Cultures producing growth typical of leptospires were examined by dark-field microscopy and inoculated intraperitoneally into golden hamsters.

Examination for brucellas

Samples of slurry were streaked on 3 replicates of albimi agar (Joint FAO/WHO Expert Committee on Brucellosis, 1958) and incubated at 37° C. 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 slurry in physiological saline were incubated aerobically on nutrient agar (Oxoid CM3) at 20° C. for 72 h. 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); 0·1 ml. volumes of appropriate dilutions of slurry in physiological saline were spread over the surface of MacConkey agar plates and incubated at 37° C. for 24 h.

pH and total solids concentration

pH was measured on the undiluted sample using a membrane electrode. When available, 40 g. of each sample was heated in a hot-air oven at 104° C. for 24 h. The residual solids were weighed and recorded as a percentage of the original weight of the wet sample.

RESULTS

Isolation, enumeration and characterization of salmonellas

Twenty strains of *Salmonella* were isolated. Twelve were identified as *S. dublin*, 4 as *S. typhimurium*, and 4 as other serotypes. These were *S. indiana*, *S. bredeney*, *S. cerro* and *S.* 4,12:d:—.

The value of various enrichment broths and the volume of sample enriched in isolating salmonellas is illustrated in Table 1.

From the 187 samples examined, salmonellas were isolated from 12 samples enriched in single-strength SBG broth; from 13 samples enriched in double-strength SBG; from 11 samples enriched in Rappaport's broth. These differences are not significant.

Of the volumes of sample examined, 10 g. was clearly the most successful. The numbers of salmonellas per 100 g. samples can be estimated approximately from the results of examination of 1×5 g. and 5×1 g. samples using Table 1 (Report, 1969) and correcting for the smaller volumes examined.

In two samples (1, 13) salmonellas per 100 g. exceeded 180: of the remaining samples, the count in three (2, 17, 18) was 160; in two (4, 5) 90; in two (3, 6) 50; in two (14, 15) 30; in three (7, 8, 19) 20; in one (9) 10. In the remaining 5 samples

Table 1. *Isolation of salmonellas in selenite brilliant green enrichment and Rappaport's broth*

Isolate No.	Selenite brilliant green broth							Rappaport's broth			Total (10)	Serotype
	Single strength			Double strength								
	1*	2*	3*	1 gm.‡	2·5 gm.‡	5 gm.‡	10 gm.‡	1*	2*	3*		
1	+	+	+	+	+	+	+	+	+	+	10	*S. dublin*
2	+	+		+	+	+	+	+	+		8	*S. dublin*
3	+	+		+	+		+	+	+		7	*S. dublin*
4	+	+	+	+	+	+	+				7	*S. dublin*
5					+	+	+	+	+	+	6	*S. dublin*
6	+			+			+	+	+	+	6	*S. dublin*
7	+	+		+	+		+				5	*S. dublin*
8	+			+	+		+	+			5	*S. dublin*
9	+			+							2	*S. dublin*
10				+			+				2	*S. dublin*
11							+				1	*S. dublin*
12							+				1	*S. dublin*
13	+	+		+	+	+	+	+	+	+	9	*S. typhimurium*
14					+	+	+	+			4	*S. typhimurium*
15						+	+	+			3	*S. typhimurium*
16							+				1	*S. typhimurium*
17	+			+	+	+	+	+	+	+	8	*S. unnamed* 4,12:d:–
18	+			+		+	+	+	+		6	*S. indiana*
19	+	+		+	+		+				5	*S. bredeny*
20							+				1	*S. cerro*
	12	7	2	13	11	9	19	11	8	5		Total (20)

+, Isolation of *Salmonella*.
*, Replicates of selenite brilliant green broth and Rappaport's broth.
‡, Wet weight of slurry enriched.

(10, 11, 12, 16, 20), using all the volumes of sample examined, salmonellas were isolated from approximately 25 g. of sample, indicating a count of between 4 and 5 per 100 g.

All strains produced gas in 1 % glucose peptone water and none were agglutinable in 1/500 neutral acriflavine.

Of eleven *S. dublin* strains biotyped according to the scheme of Walton (1972), 1 was placed in group A, 7 in group D, 2 in group F and 1 in group G. According to the scheme of Hall & Taylor (1970), seven *S. dublin* strains were included in biotype B, and 1 in biotype E, while 3 were untypable. There was thus little correlation between the two methods of typing.

Three of the *S. typhimurium* strains isolated were of phage type U165. The fourth was of phage type U239.

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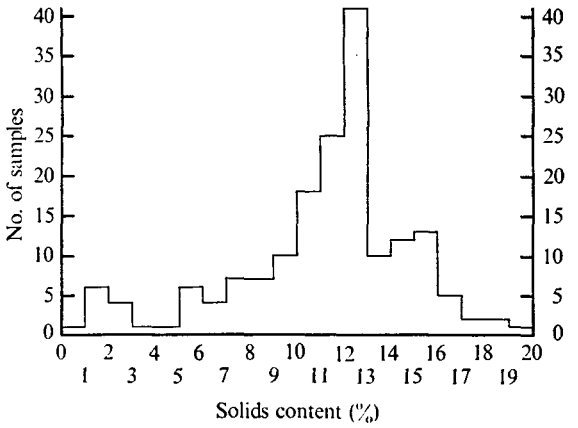

Fig. 1. The total solids concentrations of 176 samples of bovine slurry. (Two samples not included had total solids of 34·3 and 25 per cent.)

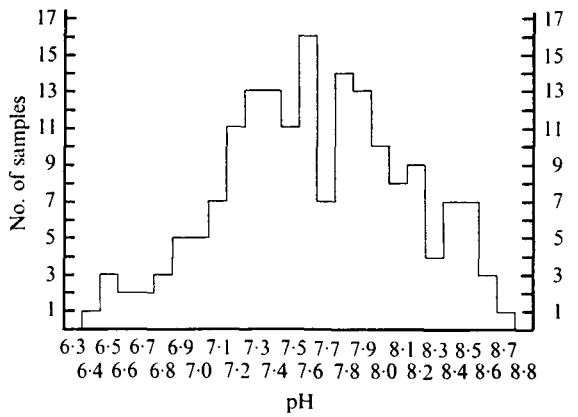

Fig. 2. The pH of 176 samples of bovine slurry (one sample not included had a pH of 5·9).

Examination for leptospires and brucellas

Sixty-four suspected leptospire colonies from 56 slurry samples were removed for further examination. All were confirmed as treponemas by dark-field microscopy.

Sixty-four hamsters inoculated with these organisms survived up to 21 days when they were killed. Antibodies to leptospires were not demonstrated in 10 hamsters examined at random. No brucellas were isolated.

Total colony count and coliform count

The total colony count per sample of slurry ranged widely, with a maximum at 10^9 organisms/g. There was a relation between the colony count and the total solids concentration. Samples with a high solids concentration had a high colony count.

The coliform count ranged from less than 10^3/g. to more than 10^6/g. with most samples containing between 10^5 and 10^6 coliforms/g.

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Total solids concentration

The total solids concentration of the samples is shown in Fig. 1. This ranged from less than 1 % (one sample) to 19 %, with a mean of 11·3 %. There were two samples outside the range at 26 % and 34 %.

The pH of samples is shown in Fig. 2. This ranged from 5·9 to 8·7 with a mean at 7·6.

DISCUSSION

The twenty strains of *Salmonella* isolated from slurry represent an incidence of 11 %. Since this is on the basis of one examination the actual contamination rate may be higher. There is no recent information on herd isolations or disease incidents with which to compare this figure, but a figure of 1 in 10 herds infected is perhaps surprisingly high. However, only small numbers of organisms were present in each case. This could indicate either that few animals in each herd were excreting or that the slurry had aged and the numbers of salmonellas declined. Jones (to be published) has shown that salmonellas decline by 90 % after 2–4 weeks of storage. The systems examined were chosen at random and therefore represented a variety of times between emptying.

The assumption that all the salmonellas found in the slurry originated from cattle excreta may be invalid. MacDonald & Brown (1974) have shown that the phage-types of *S. typhimurium* isolated in this survey are common in wild birds, and the infection may have been introduced from this source rather than from the cattle. Nevertheless the incidence of *S. dublin* is high and it is unlikely that these organisms originated from any source other than the cattle.

The dose of salmonellas required to infect cattle varies with different serotypes and strains and is not established. Taylor (1973) demonstrated that a strain of *S. dublin* failed to infect grazing calves consuming grass sprayed with slurry containing 10^5 *S. dublin*/ml. The numbers of *S. dublin* isolated are considered unlikely to cause infection under normal husbandry conditions. However, the possibility of the organism multiplying in the alimentary canal of apparently non-affected animals should be considered. Such animals could possibly excrete sufficient organisms onto pasture for the pasture to constitute a hazard to other grazing animals. The number of *S. typhimurium* required to cause an infection is probably lower than with *S. dublin* and therefore *S. typhimurium* may present a hazard at this level.

The salmonellas isolated were aerogenic and smooth, and thus not of reduced virulence according to Walton & Lewis (1971). The live calf vaccine strain (S51) which belongs to Walton's biotype E was not isolated, supporting Hall & Taylor's (1970) observation that this strain has not been widely disseminated.

It is interesting to note that the proportions of serotypes isolated (*S. dublin* 60 %, *S. typhimurium* 20 %, other serotypes 20 %) correspond with those isolated from incidents of disease diagnosed at the MAFF Laboratories (*S. dublin* 79 %, *S. typhimurium* 18·5 %, other serotypes 2 %) (Wray & Sojka, 1972).

The failure to isolate brucellas or pathogenic leptospires is perhaps not sur-

prising. Albimi agar did not inhibit the growth of many contaminants, particularly fungi, which tended to obscure bacterial growth. It was known that brucella-positive herds were included in the survey but the principal source of brucellas would be from aborted material which would probably not gain access to a slurry system.

Many strains of leptospires were isolated but their inability to kill hamsters indicates that they were saprophytes. Diesch, Pomeroy & Allred (1971) experienced difficulty in re-isolating *Leptospira pomona* seeded into a model field oxidation ditch.

Rankin & Taylor (1969) reported on the pH and dry-matter content of 16 samples of bovine slurry. Their samples had an average pH of 6·7 and a maximum dry matter content of 12·4 %. Figs. 1 and 2 illustrate that the values measured in the present survey often differed from those of Rankin & Taylor. Slurry is usually considered to be a mixture of faeces, urine and water and therefore many of the total solids values shown in Fig. 1 may be considered too high for the samples to be treated as slurry. However, the systems of storage examined included tanks, compounds and lagoons. The dry-matter content would thus vary depending on whether rain water was allowed to gain access or evaporation take place, as well as with the volume of water used in hosing down of yards and channels. The values measured are thus considered to be an accurate reflexion of conditions prevailing during storage and spreading of slurry.

Jones (to be published) has shown both the pH and solids concentration of slurries to be important factors in determining the survival of *S. dublin*. Many previous estimates of the survival of *S. dublin* assessed in very dilute slurries may therefore be too short.

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