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# The occurrence of salmonellas, mycobacteria and pathogenic strains of *Escherichia coli* in pig slurry

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## SUMMARY

Ninety-eight samples of pig slurry from 54 farms were examined for the presence of salmonellas, porcine enteropathogenic strains of haemolytic *Escherichia coli* and mycobacteria. Salmonellas were isolated from 12 farms (22%) and enteropathogenic *E. coli* from 13 farms (24%). Pathogenic mycobacteria were not isolated.

Salmonellas were isolated from 7 of 16 farms (44%) stocked with 'minimal disease' pigs compared with only 5 of 38 farms (13%) stocked with conventionally reared pigs. Conversely enteropathogenic coliforms were isolated from 3 of 16 farms (19%) stocked with 'minimal disease' pigs compared with 10 of 38 farms (26%) stocked with conventionally reared pigs.

## INTRODUCTION

In a survey of slurry from dairy and beef farms in England and Wales, Jones & Matthews (1975) isolated salmonellas from 20 (11%) of 187 slurry samples. They concluded that although the serotypes isolated corresponded closely to serotypes implicated in disease in cattle the numbers of organisms contained in the slurry were small and unlikely to create a significant hazard to grazing animals when the slurry was applied to pasture. However, as the intensification of livestock farming continues it is becoming increasingly common for slurry from pigs to be spread on pasture used for grazing by cattle. Although *Salmonella choleraesuis* and *S. typhimurium* are mainly responsible for salmonellosis in pigs (Sojka, Wray, Hudson & Benson, 1975) many other serotypes may occur without obvious clinical signs of disease. These serotypes may be excreted in the faeces of 'carrier' animals and could if present in pig slurry cause disease in cattle grazing pasture over which it is spread. The incidence of salmonellas in pigs has been assessed at approximately 12–14% on the basis of examination of faeces and mesenteric glands (PHLS Working Group, Skovgaard & Nielsen, 1972). There are, however, no current data on the rate of infection of pig herds and thus on the number of pig slurry systems which may contain salmonellas.

Jones & Hall (1975) suggested that examination of slurry to detect *Salmonella* infection in a herd may be a rapid, simple and inexpensive alternative to examination of faeces from individual animals and may be particularly useful in the

detection of herds containing animals which excrete infrequently and which may not be readily detected by conventional swabbing techniques.

The purpose of the work described here was to examine a number of slurry systems to assess the degree to which they were contaminated with salmonellas, and to determine whether examination of slurry may be useful in detecting the presence of mycobacteria and enteropathogenic stains of *Escherichia coli* in a pig herd.

## MATERIALS AND METHODS

### *Pig slurry samples*

Samples of pig slurry from farms in Berkshire, Oxfordshire and Hampshire were collected into 100 ml. bottles and returned immediately to the laboratory for analysis. The owner or manager of each farm was asked whether his herd was stocked with 'minimal disease' or 'conventional' pigs and figures produced later are based on the answer.

A total of 98 samples from 54 farms were taken, and were divided into 60 samples from 38 farms stocked with conventional pigs and 38 samples from 16 farms stocked with 'minimal disease' pigs.

### *Isolation, enumeration 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 millilitres of slurry 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 CM32) 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 (1962) and serologically according to the method of Kauffmann (1972).

The concentrations of salmonellas in samples were estimated by spreading 0·1 ml. volumes of appropriate dilutions of slurry in saline over the surface of modified brilliant green agar (as above); the plates were incubated at 37° C. and colonies agglutinating in *Salmonella* polyvalent O serum (Wellcome laboratories) were counted.

### *Isolation of mycobacteria*

Stuart's basal medium (Stuart, 1965) with the addition of 10 ml. egg yolk emulsion (Oxoid SR47), 4 ml. 9% bovine albumen (Armour laboratories), penicillin* (100 units/ml.) and amphotericin B† (50 μg./ml.) to 96 ml. of basal medium was used for the isolation of mycobacteria.

* Benzylpenicillin B.P. Glaxo Laboratories.

† Fungizone E. R. Squibb and Sons Inc., New York.

Approximately 10 ml. of sample was centrifuged at 800 *g* for 15 min. and 1 g. of the deposit was added to 10 ml. of 0·26% benzalkonium chloride in 10% trisodium phosphate. The resultant suspension was filtered through muslin, incubated at 37° C. for 30 min. and re-centrifuged at 800 *g* for 15 min. After centrifugation the deposit was resuspended in 2 ml. physiological saline containing amphotericin B (1000 μg./ml.) and neutralized with normal hydrochloric acid. The suspension was distributed on 5 slopes of isolation medium (as above) and 5 slopes of isolation medium with the addition of 3000 μg./ml. crude mycobactin (Stuart, 1965). The slopes were incubated at 37° C. and examined at intervals for up to 6 months.

*Isolation of porcine enteropathogenic strains of haemolytic* Escherichia coli

0·05 ml. of slurry was spread on 2 blood agar plates (bovine blood, 7·5%). An antibiotic multodisk (Oxoid 30-3G) was placed on the surface of one of the plates and both were incubated at 37° C. for 18 h. Colonies resembling *Escherichia coli* in colony morphology and surrounded by a zone of haemolysis were identified serologically according to Sojka (1965).

*Antibiotic sensitivity of* Salmonella *and* E. coli *strains*

Strains of *Salmonella*, *E. coli* and a standard strain of *E. coli* (NCTC 10418) were tested on blood agar (bovine blood, 7·5%) for sensitivity to ampicillin (2 and 25 μg.), cephaloridine (5 and 25 μg.), chloramphenicol (10 and 50 μg.), colistin sulphomethate (50 μg.), colistin (200 μg.), erythromycin (10 μg.), framycetin (100 μg.), fucidin (10 μg.), furazolidone (100 μg.), gentamycin (10 μg.), kanamycin (30 μg.), lincomycin (2 μg.), methicillin (10 μg.), naladixic acid (30 μg.), neomycin (10 and 30 μg.), nitrofurantoin (200 μg.), novobiocin (5 and 30 μg.), oxytetracycline (5 μg.), penicillin G (2 and 5 units), polymyxin B (300 units), spectinomycin (25 μg.), streptomycin (10 and 25 μg.), tetracycline (10, 30 and 50 μg.) (Oxoid, single disk).

Plates with methicillin disks were incubated at 30° C. and other plates at 37° C. for 18 h. and zones of inhibition measured.

*pH and total solids content*

pH was measured on the undiluted sample using a membrane electrode. Ten grams 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*

Salmonellas were isolated from 19 samples of slurry taken from twelve farms, including 5 samples from 5 farms stocked with 'conventionally' reared pigs and 14 samples from 7 farms stocked with 'minimal disease' pigs.

The strains isolated and the value of the various enrichment broths in their

Table 1. *Isolation of salmonellas from pig slurry*

| Farm no. | Selenite BGB* 1 | Selenite BGB* 2 | Rappaport broth 1 | Rappaport broth 2 | BGMB† 37 | BGMB† 43 | Salmonella plate count cfu/ml. | Serotype |
|---|---|---|---|---|---|---|---|---|
| 1 | + | − | + | − | − | − | | *S. typhimurium* DT17 |
| 2 | + | − | + | − | − | − | | *S. typhimurium* DT8 |
| 3 | + | + | + | − | − | − | | *S. indiana* |
| 4 | + | − | + | − | + | − | | *S. panama* |
| 5 | + | + | + | + | + | + | | *S. derby* |
| 6 | + | − | + | − | − | − | $2 \times 10^3$ | *S. eimsbuettel* |
| 7 | − | − | + | − | − | − | | *S. senftenberg* |
| 8 | + | − | − | − | − | − | | *S. livingstone* |
| 9 | − | − | + | + | − | − | | *S. livingstone* |
| 10 | − | − | − | − | − | − | $2 \times 10^3$ | *S. give*‡ |
| 11 | + | + | − | − | + | − | | *S. livingstone* |
| | − | − | + | − | − | − | | *S. kentucky* |
| | + | + | + | + | + | + | | *S. schwarzengrund* |
| 12 | − | − | + | + | + | + | | *S. give* |
| | + | − | + | + | + | + | $5 \times 10^3$ | *S. derby* |
| | − | − | + | + | + | + | | *S. derby* |
| | − | − | + | + | + | + | | *S. derby* |
| | − | − | + | + | + | + | | *S. derby* |
| | − | − | − | − | + | − | | *S. derby* |
| | 10 | 4 | 15 | 8 | 10 | 7 | | Total 19 |

* Selenite brilliant green broth.

† Brilliant green MacConkey broth, incubated at 37 and 43° C.

‡ Isolated from brilliant green agar used for plate count.

Two replicates each of selenite brilliant green broth and Rappaport broth were used, as shown.

Table 2. *Isolation of enteropathogenic strains of* Escherichia coli *from pig slurry*

| Farm no. | OK group isolated |
|---|---|
| 2 | Abbotstown [O149:K91(B), K88ac(L)] |
| 3 | E57 [O138:K81(B)] |
| 12 | E57 [O138:K81(B)] |
| | E57 [O138:K81(B)] |
| | G1253 [O147:K89(B), K88ac(L)] |
| 13 | E57 [O138:K81(B)] |
| 14 | E57 [O138:K81(B)] |
| | G205 [O8:K87(B), K88ac(L)] |
| 15 | E65 [O45:K'E65'] |
| 16 | G4/66 [O45:K'E65'K88ac(L)] |
| 17 | E6811 [O141:K85ab(B)–K85ac(L)] |
| 18 | E4 [O139:K82(B)] |
| 19 | E4 [O139:K82(B)] |
| 20 | E4 [O139:K82(B)] |
| 21 | E4 [O139:K82(B)] |
| 22 | E4 [O139:K82(B)] |
| 13 Total | 16 |

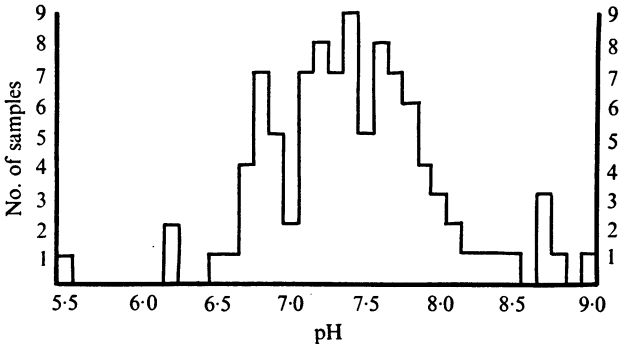


Fig. 1. The pH of 98 samples of pig slurry.

isolation are illustrated in Table 1. From the 98 samples examined, salmonellas were isolated from 15 samples enriched in Rappaport broth; from 10 samples enriched in SBG broth; from 10 samples enriched in brilliant green MacConkey broth at 37° C. and from 7 samples enriched in brilliant green MacConkey broth at 43° C.

No more than one serotype of *Salmonella* was isolated per sample though more than one serotype was isolated from 2 of the farms from which more than one sample of slurry was taken (Table 1).

Of the 19 samples from which salmonellas were isolated only 3 contained sufficient to be counted by the plate count method and these are also shown in Table 1.

*Isolation of mycobacteria*

Four strains of mycobacteria were isolated but were identified as rapid-growing non-pathogenic strains.

*Isolation of porcine enteropathogenic strains of haemolytic* Escherichia coli

Enteropathogenic strains of *E. coli* were isolated from 16 samples of slurry taken from 13 farms (Table 2), including 11 samples from 10 farms stocked with conventionally reared pigs and 5 samples from 3 farms stocked with 'minimal disease' pigs.

*Antibiotic sensitivity of* Salmonella *and* E. coli *strains*

The standard strain of *E. coli* (N.C.T.C. 10418) was resistant to cloxacillin, erythromycin, fucidin, lincomycin, methicillin, novobiocin, oxytetracycline and penicillin G. All isolated strains of *Salmonella* and *E. coli* tested were similarly resistant with the exception of 13 *Salmonella* strains sensitive to penicillin G (2 units), 6 *E. coli* strains sensitive to penicillin G (5 units), 6 *Salmonella* strains sensitive to oxytetracycline and 4 *E. coli* strains sensitive to oxytetracycline.

Four *Salmonella* strains (*S. senftenberg*, *S. indiana*, *S. derby* and *S. typhimurium*

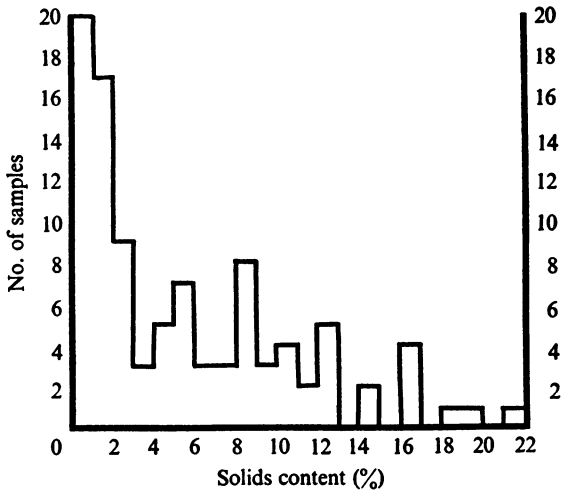


Fig. 2. The solids content of 98 samples of pig slurry.

DT8) and 7 *E. coli* strains (E4 (4 strains) E57 (3 strains)) were resistant to tetracycline (50 $\mu$g.) and 2 *E. coli* strains (G4/66(1), E4(1)) were resistant to streptomycin (25 $\mu$g.).

*pH and total solids content*

The pH of samples is shown in Fig. 1. This ranged from 6·2 to 9·0 with a mean at 7·4. The total solids content of samples is shown in Fig. 2. This ranged from 0·3% to 21·9% with a mean of 5·5%.

## DISCUSSION

The isolation of salmonellas from slurry from 12 of 54 farms represents an incidence of infection of 22%. This figure is based on one sampling and the actual incidence might have been greater if repeated samples had been taken. It should, however, be stressed that the farms sampled were restricted to the counties of Berkshire, Oxfordshire and Hampshire and the results may not indicate the national incidence of infection with *Salmonella* in pig herds.

If examination of slurry gives an accurate reflexion of the rate of infection in herds then it appears that infection with *Salmonella* in pigs is higher than in cattle. Jones & Matthews (1975) isolated salmonellas from 11% of slurry samples from dairy and beef herds in England and Wales. It is also of interest that although none of the serotypes isolated appeared to be associated with disease amongst the pigs the largest proportion of isolations was made from farms stocked with 'minimal disease' pigs – 7 of 16 farms (44%) compared with only 5 of 38 farms (13%) stocked with conventionally reared pigs. This may have been more related to the size of the units rather than to their 'minimal disease' status. The 'minimal disease' herds were usually larger than conventionally stocked herds and the slurry was thus derived from more animals. It is possible that since such large units buy more feed and feed additives there is a greater chance of acquisition of salmonellas from this source.

Although none of the serotypes isolated were shown to be associated with disease amongst the pigs all are presumably capable of causing disease amongst cattle. Thus although there is little chance of spreading salmonellosis amongst pigs by the use of a slurry system a danger may exist when slurry from pig units is spread on pasture which will subsequently be grazed by cattle. Although the number of salmonellas contained in the slurry was generally low, these results indicate the wisdom of storing pig slurry for at least a month before spreading on pasture (Jones, 1976).

This survey also revealed a high incidence of enteropathogenic coli in pigs. The OK groups isolated were those frequently found in association with *E. coli* diarrhoea (Abbotstown, G1253, G4/66, G205), with both 'Oedema disease' and *E. coli* diarrhoea especially in the post-weaning period (E57, E6811) or with 'Oedema disease' alone (E65, E4) (Sojka, 1965). Whereas the enteropathogenic coli were isolated from 24% of farms they were present in only 19% of 'minimal disease' herds compared with an isolation rate of 26% from conventionally reared herds.

The failure to isolate pathogenic mycobacteria is perhaps not surprising. The method of enrichment for these organisms is less sensitive than for salmonellas or coliforms. In addition most of the herds sampled were kept indoors continually and it is probable that these units are less likely to contain mycobacterial infection than herds which are kept outside (Matthews, 1969).

The tests of antibiotic resistance were carried out only on the relatively few salmonellas and coliforms isolated and may therefore not be representative of resistance among these organisms in general; however, the level of resistance shown was low. Similar results were obtained by Sojka, Slavin, Brand & Davies (1972) who examined 74 strains of *Salmonella* isolated from pigs, although the resistance to streptomycin reported by these authors was not found in the present survey.

Jones & Matthews (1975) reported on the pH and dry matter content of 176 samples of cattle slurry. Their samples had an average pH of 7·6 and an average dry-matter content of 11·3%. The average pH value of the samples in the present survey (7·4) was similar to the value for cattle slurry while the average dry matter content was lower (5·5%). This is probably a reflection of the differences between the methods of collecting and storing pig and cattle slurry. Pig slurry is usually stored in tanks which also collect urine while the majority of cattle slurry systems rely on storage in an outdoor lagoon or storage compound from which water is lost by drainage or evaporation.

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