J. Hyg., Camb. (1975), **74**, 65
Printed in Great Britain

The effect of storage in slurry on the virulence of *Salmonella dublin*

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(*Received* 10 *July* 1974)

SUMMARY

The mouse was used as a model to determine whether storage of *Salmonella dublin* in slurry and in broth reduces the virulence of the organism. No reduction in virulence of *S. dublin* stored in slurry for 36 days or in maintenance broth for 70 days was observed. The disease hazard involved in pasture-spreading of slurry contaminated with salmonellas is related to factors other than virulence.

INTRODUCTION

It is common practice to mix excreta from cattle with water prior to disposal as a slurry. This is usually stored for periods of from a few hours to 2-3 years before being spread on pasture which may subsequently be grazed by cattle.

Since slurry does not compost, pathogenic micro-organisms which are capable of surviving the period of storage may be a hazard to the health of grazing animals.

Jones & Matthews (1975) demonstrated the presence of salmonellas in 11% of bovine slurry samples from farms in England and Wales and it is known that these organisms may survive for a considerable time in the slurry. Rankin & Taylor (1969) and Findlay (1971) have shown that *S. dublin* may survive for more than five months in bovine slurry but it is not known if storage affects pathogenicity. The work described in this paper was an attempt to answer this question.

MATERIALS AND METHODS

Salmonella dublin

An aerogenic smooth strain of *S. dublin* (3246) isolated by M. H. Hinton from a case of abortion in a dairy cow was used.

Mice

White BSVS male mice were used at an average weight of 21 g. They were housed one animal per cage. All mice received 0·25 ml. of inoculum delivered into the stomach from a syringe needle with a 'pear drop' end.

Bacto-tryptose broth

Bacto-tryptose (BT) broth was prepared according to the formula: bacto-tryptose (Difco), 20 g., sodium chloride 5 g., β-sodium glycerophosphate 2 g., glucose 1 g., distilled water 1000 ml., and had a pH of 7·4.

Slurry

Slurry was collected from a lagoon at one of the Institute's dairy farms and represented the bulked excreta from a herd of over 100 Friesian cows. The slurry was centrifuged at 1000 ***g*** for 2 min. to remove fibre. The solids content and pH of the batches used are given in the text.

Each batch of slurry was examined for the presence of salmonellas before use by plating on Oxoid modified brilliant green agar (with the addition of 120 mg./l. sulphadiazine [BDH]) and by enrichment of 1 g. amounts of slurry in Difco selenite brilliant green enrichment broth (SBG) and in Rappaport's broth (Rappaport, Konforti & Navon, 1956). The modified brilliant green agar was incubated at 37° C. and examined after 24 and 48 hr. The SBG and Rappaport's broths were incubated at 43 and 37° C. respectively and plated onto modified brilliant green agar (as above) after 24 and 48 hr. incubation.

Storage of slurry and of bacto-tryptose broth

Slurry and BT were stored in 200 ml. volumes in a water bath at $10 \pm 0{\cdot}1°$ C.

Estimation of S. dublin *in inocula*

The concentration of *S. dublin* in inocula was estimated by the 'surface viable count by spreading' method of Cruickshank (1968). Volumes of 0·1 ml. of appropriate dilutions in saline were spread over the surface of modified brilliant green agar (as above) and incubated at 37° C. for 24 hr. The average count from 5 replicates was recorded.

Experiment 1

Determination of pathogenicity of S. dublin *for mice*

Four groups of 12 mice received 0, $2{\cdot}5 \times 10^3$, $2{\cdot}5 \times 10^5$ and $2{\cdot}5 \times 10^7$ *S. dublin* cells in slurry and were examined daily for 18 days. Deaths of mice were recorded daily. The slurry had a total solids content of 1·3 % and the pH was 8·0.

Experiment 2

Determination of the effect of storage in slurry and bacto-tryptose broth on the virulence of S. dublin

An 18-hr. BT culture containing $1{\cdot}4 \times 10^9$ *S. dublin*/ml. was serially diluted in BT and in slurry to produce inocula containing $1{\cdot}4 \times 10^4$ *S. dublin*/ml. in BT and slurry respectively. Each was used to infect orally two groups of 50 mice as previously described. A third group of 50 mice received slurry without *S. dublin*. The mice were examined daily for 18 days, after which time survivors were killed.

The 18-hr. BT culture was also used to infect slurry with *S. dublin* prior to storage. It was diluted in slurry to give a final concentration of 1×10^7 *S. dublin*/ml. in 200 ml. of slurry. This was stored as previously described. Equivalent volumes of uncontaminated slurry and of the original BT culture were stored in the same manner. The survival of *S. dublin* in the stored slurry and stored BT culture was examined by plate counts as described above.

After 36 days the slurry contained $1{\cdot}4 \times 10^4$ *S. dublin* per ml. which corresponded to the inoculum administered to the 50 mice described above. The BT broth contained $1{\cdot}5 \times 10^8$ *S. dublin*/ml. This was diluted to contain $1{\cdot}4 \times 10^4$ *S. dublin*/ml. by serial dilution in filter-sterilized volumes of the original broth.

Two groups of 50 mice were inoculated with the stored slurry and stored BT broth containing *S. dublin* as previously described. A third group of 50 mice received the stored uncontaminated slurry. The dose of *S. dublin* administered prior to and after storage was thus the same.

Experiment 3

Determination of the effect of prolonged storage in bacto-tryptose broth on the virulence of S. dublin

The BT culture was stored an additional 34 days (total storage 70 days), then diluted to contain $1{\cdot}5 \times 10^4$ *S. dublin*/ml. and administered to a group of 50 mice. A second group of 50 mice received an overnight BT culture of *S. dublin* diluted in BT to contain $1{\cdot}5 \times 10^4$ *S. dublin*/ml. In addition, 2 groups of 25 mice received either stored or fresh BT broth, filtered to remove bacterial cells.

Examination of mice for S. dublin

Mice which died or which were killed after the termination of experiments were examined for the presence of *S. dublin*. Stomach, intestinal and rectal contents were mixed and examined by inoculation of modified brilliant green agar and SBG as previously described.

RESULTS

Experiment 1

Determination of pathogenicity of S. dublin *for mice*

The cumulative mortality of the 4 groups of mice is shown in Table 1. A dose of $2{\cdot}5 \times 10^3$ *S. dublin*/mouse produced 50 % mortality in 17 days and was considered a suitable dose for further experiments to determine the effect of storage on the virulence of *S. dublin*. There were no deaths in the group of mice which received slurry not contaminated with *S. dublin*.

Experiment 2

Effect of storage on the virulence of S. dublin

The number of mice dying within 18 days after administration of fresh or stored *S. dublin* is shown in Table 2. There was no decrease in the mortality of mice in

Table 1. *Response of mice to oral administration of* Salmonella dublin *in slurry*

Time after infection (days)	Cumulative mortality in 12 mice given slurry containing *S. dublin* in the following numbers			
	$2{\cdot}5 \times 10^7$	$2{\cdot}5 \times 10^5$	$2{\cdot}5 \times 10^3$	None
7	2	1	1	0
8	3	4	1	0
9	6	7	3	0
10	8	7	3	0
11	10	8	4	0
12	11	9	4	0
13	12	9	5	0
14	12	9	5	0
15	12	9	5	0
16	12	9	5	0
17	12	9	6	0
18	12	9	7	0

Table 2. *Effect of storage in slurry or in bacto-tryptose broth, on the virulence of* S. dublin

Time after infection (days)	Cumulative mortality in 50 mice given:					
	Freshly prepared *S. dublin*† in slurry	Stored *S. dublin*† in slurry	Freshly prepared *S. dublin*† in BT*	Stored *S. dublin*† in BT*	Fresh slurry	Stored slurry
5	0	1	0	0	0	0
6	1	2	0	0	0	0
7	2	4	2	2	0	0
8	3	6	10	7	0	0
9	6	7	12	10	1	0
10	9	13	14	19	1	0
11	11	16	16	21	1	1
12	14	18	17	25	1	1
13	16	20	17	25	1	1
14	18	20	17	26	1	1
15	18	20	17	26	1	1
16	18	21	17	27	1	1
17	18	21	17	27	1	1
18	18	21	17	27	1	1

* BT = Bacto-tryptose broth. † The dose of *S. dublin* employed was $2{\cdot}5 \times 10^3$.

groups given *S. dublin* stored either in slurry or in BT. On the contrary, the mortality among mice in groups which had received stored *S. dublin* appeared to be slightly increased, but this increase was not significant. Storage had no effect on the incubation period of the disease.

Experiment 3

Effect of prolonged storage in bacto-tryptose broth

The number of mice dying within 18 days after administration of fresh *S. dublin* or *S. dublin* stored for 70 days in BT broth is shown in Table 3. Storage for an

https://doi.org/10.1017/S0022172400046726 Published online by Cambridge University Press

Table 3. *Effect of prolonged storage in bacto-tryptose broth on the virulence of* S. dublin

Time after infection (days)	Cumulative mortality in 50 mice given: Freshly prepared *S. dublin* in BT†	Cumulative mortality in 50 mice given: Stored *S. dublin*‡ in BT†	Cumulative mortality in 25 mice given: Freshly prepared sterile* BT†	Cumulative mortality in 25 mice given: Freshly prepared sterile* slurry
7	1	1	0	0
8	7	8	0	0
9	21	14	0	0
10	27	24	0	0
11	29	27	0	0
12	29	29	0	0
13	30	31	0	0
14	30	32	0	0
15	30	33	0	0
16	30	33	0	0
17	30	33	0	0
18	31	33	0	0

* *S. dublin* removed by membrane filtration.
† BT = Bacto-tryptose broth.
‡ The dose of *S. dublin* given was $2{\cdot}6 \times 10^3$.

additional 34 days in BT did not reduce the virulence of *S. dublin* or alter the incubation period of the disease. There again appeared to be a slightly higher mortality in mice given stored *S. dublin* rather than freshly cultured *S. dublin*, but this was not significant.

Post-mortem examination of mice

S. dublin was isolated by direct plating from all mice which received *S. dublin* and subsequently died. *S. dublin* was isolated from only three survivors of the inoculated mice. *S. dublin* was not isolated from any of the control mice.

DISCUSSION

No apparent reduction in virulence was noted in *S. dublin* stored either in slurry for up to 36 days or BT for up to 70 days. Mortality was slightly higher in groups of mice which had received stored rather than freshly prepared *S. dublin*. However, since the experiment was spread over 70 days there was difficulty in obtaining complete standardization of broth and mice and small variations in these may have been responsible for any slight variation in the response of mice to the doses given.

It appears, however, that *S. dublin* survives in slurry for up to a month without loss of virulence. Thus in assessing the hazards of a slurry system to animal health, factors other than the continued virulence of salmonellas must be considered. The disease risk associated with infected slurry is presumably related to the number of pathogenic organisms present rather than to their virulence. Jones & Hall (to be published) were unable to infect pregnant heifers with a dose of 10^7 *S. dublin*

intravenously. This figure may not reflect the infective dose of this organism under natural conditions but it certainly appears that large numbers of *S. dublin* are required to cause an infection. 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 degree of infection of slurry systems may never reach this figure. Jones & Matthews (1975) have shown that when salmonellas are isolated from slurry systems thay are usually present in small numbers (less than 100 per 100 ml.).

Thus although *S. dublin* will remain capable of causing disease, the risk to other animals of contaminated slurry will depend on other factors including the number of animals in a herd excreting the organism, the dilution of infected excreta with non-infected excreta, the period for which the slurry is stored prior to spreading on pasture and the time elapsing before infected pasture is grazed.

I am grateful to Miss J. Kirby for assistance with the maintenance and examination of mice.

REFERENCES

CRUICKSHANK, R. (1968). *Medical Microbiology*, 11th ed., p. 872. Edinburgh and London, E. & S. Livingstone.

FINDLAY, C. R. (1971). The survival of *Salmonella dublin* in cattle slurry. *Veterinary Record* **89**, 224–5.

JONES, P. W. & MATTHEWS, P. R. J. (1975). Examination of slurry from cattle for pathogenic bacteria. *Journal of Hygiene* **74**, 57–64.

RANKIN, J. D. & TAYLOR, R. J. (1969). A study of some disease hazards which could be associated with the system of applying cattle slurry to pasture. *Veterinary Record* **85**, 578–81.

RAPPAPORT, F., KONFORTI, N. & NAVON, BETTY (1956). A new enrichment medium for certain salmonellae. *Journal of Clinical Pathology* **9**, 261.

TAYLOR, R. J. (1973). A further assessment of the potential hazard for calves allowed to graze pasture contaminated with *S. dublin* in slurry. *British Veterinary Journal* **129**, 354.