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THE PERSISTENCE OF SALMONELLA TYPHI MURIUM IN VARIOUS TYPES OF MANURE WITH AND WITHOUT ADMIXTURE OF SILAGE EFFLUENT*

By

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GUDDING, ROAR: *The persistence of Salmonella typhi murium in various types of manure with and without admixture of silage effluent.* Acta vet. scand. 1975, *16*, 115—125. — Salmonella typhi murium, NVH 550 added in a concentration of 5×10^5 bacteria per ml could be detected in liquid manure and semi-solid manure, after 20 weeks and 12 weeks, respectively, at 10°C. In urine and solid manure, also held at 10°C, the test bacteria persisted for about 5 weeks. Salmonella typhi murium could not be isolated from solid manure held at 45°C for 14 days.

Addition of silage effluent to final concentrations of 20, 40 and 60 % reduced the survival times in mixtures with liquid manure, while the opposite effect was observed in the mixtures of silage effluent and semi-solid manure or urine.

Inhibitory substances against Salmonella typhi murium or Sarcina lutea NVH 546 could not be demonstrated in any of the mixtures by the agar diffusion test.

The effects of environmental factors on the survival time of Salmonella typhi murium in different types of manure are discussed.

s a l m o n e l l a; m a n u r e.

During the last decade the consistency and nature of manure has gradually become more humid. This alteration is due to several factors, such as more intensive systems of animal management, less or no use of bedding and more compact store rooms for manure.

A consequence of the replacement of traditional farm-yard manure with a more liquid manure is an increased risk for diseases of toxicological or microbiological origin.

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During mixing of the liquid manure, toxic gases like hydrogen sulfide may escape from the manure and cause intoxications of animals and man. In Norway, some cattle and several pigs are lost annually, and farm workers have also been intoxicated, in 1 case, even fatally.

Several workers have studied the persistence of pathogenic bacteria in different types of liquid manure, and though there are great variations, the survival times are generally in the range of 1 to 7 months (*Blum* 1968, *Strauch & Hahn* 1968, *Burrows & Rankin* 1970, *Best et al.* 1971, *Jeffrey* 1971, *Findlay* 1972, *Tannock & Smith* 1972).

The potential risk for animal and human health is also associated with the way of disposing of the liquid manure. On many farms the spreading of manure on pastures is the only alternative for recycling the animal faecal waste because arable land is not available. Spreading of liquid manure on fields which are to be used for cattle grazing may lead to an uncontrolled distribution of different microorganisms (*Jack & Hepper* 1969 and *Rankin & Taylor* 1969).

In Norway, a new Act has prohibited discharge of wastes into rivers, streams or water courses (*Lov om vern mot vannforurensning* 1970). Due to a very high content of organic matter, silage effluent is a highly polluting fluid, and this Act means that silage effluent has to be taken care of and used, or disposed of in new ways. An alternative which has been approbated by Norwegian authorities, is storing the silage effluent in the tank for liquid manure.

This procedure seems to have no disadvantages if the tank has been emptied recently. However, the consequences of adding silage effluent to liquid manure which has been stored for some time, have not been sufficiently investigated. Toxic gases may escape during the mixing and the acid silage effluent lowers the pH value of the mixture, transforming any water-soluble HS-ions present to gaseous H_2S. The mixing of silage effluent with manure may also have consequences for the persistence of pathogenic bacteria.

The aim of the present work was to study the ability of Salmonella typhi murium to survive in mixtures of silage effluent and liquid manure, semi-solid manure and urine. In addition, the purpose of the investigation was to register and evaluate the effects of some biological and chemo-physical factors on the

persistence of pathogenic bacteria in different types of animal faecal waste.

MATERIAL AND METHODS

Assay material

Samples of silage effluent, liquid manure, semi-solid manure and urine were obtained via Norsk Fórkonservering A/S. There had been no addition of water to the liquid manure. The difference between semi-solid and liquid manure was mainly the supplement of urine to the faeces in the latter. Bedding was very scarce in both types of manure so the consistency of the semi-solid manure was fairly humid. The urine samples were collected from a urine tank and a small addition of faeces to the urine was unavoidable.

The samples of the 3 types of animal waste were each mixed with increasing amounts of silage effluent. The final concentrations of silage effluent in the mixtures were 20 %, 40 % and 60 %. In addition, urine, liquid manure, semi-solid manure and solid manure with bedding but without any supplement, were included in the investigation.

The content of dry matter in the samples was as follows: Liquid manure, 10.9 %; semi-solid manure, 17.3 %; solid manure, 25.5 %; urine, 1.5 % and silage effluent, 6.2 %.

Salmonella typhi murium NVH 550 was added the samples to a final concentration of 5×10^5 bacteria per ml mixture. The storage temperature during the experiments was 10°C. The samples with solid manure were also stored at 20°C and 45°C.

Bacteriological examinations

The enumeration of Salmonella organisms in the samples was performed by the Most Probable Number method (*McCrady* 1915). Five samples of 3 tenfold dilutions were inoculated onto an enrichment broth, and subinoculated onto a solid medium after 1, 2 and 3 days of incubation at 37°C. K-tetrathionate broth was used as an enrichment medium and bromo-thymol blue-lactose medium as the solid medium. Questionable colonies were subinoculated on TSI medium and urea medium, and final verification was carried out by serological examinations.

The total count of bacteria in each sample was determined by a standard plate count method using a medium with the following ingredients: Peptone, 10 g; Beef extract, 5 g; NaCl, 5 g;

Agar, 15 g. Distilled water was added to 1000 ml. The incubation conditions were as follows: 45°C for 2 days, 30°C for 3 days, 20°C for 3 days and 10°C for 7 days.

Inhibitory substances

The agar diffusion test was used for testing the antibacterial effect of the samples against Salmonella typhi murium and Sarcina lutea NVH 546. The test bacteria were mixed with beef peptone medium after cooling to 45°C, and the samples were applied into wells punched in the solid agar.

pH measurement

The pH values of the samples were recorded on a pH-Meter, Type PHM 28*.

RESULTS

The test series were repeated twice, and the results given in this paper were obtained during the first test series.

The survival times of Salmonella typhi murium in the different samples are shown in Fig. 1. The reduction in the number of viable inoculated bacteria was slower in liquid manure than in liquid manure with increasing concentrations of silage effluent (Figs. 1 a and b). Salmonella typhi murium could be detected in liquid manure, and in liquid manure with silage effluent (40:60), 20 weeks and 12 weeks, respectively, after the inoculation.

In the samples originating from semi-solid manure, the addition of silage effluent gave a prolongation of the time when the test bacteria were detectable. The survival times were 12 weeks and 16 weeks, respectively, for semi-solid manure without, and with, silage effluent (40:60) (Figs. 1 c and d).

Salmonella typhi murium numbers declined rapidly in urine, and the supplement of increasing quantities of silage effluent gave a marked increase in the time of survival, ranging from 5 weeks in the sample with urine to 14 weeks in the sample with urine and silage effluent (40:60) (Figs. 1 e and f).

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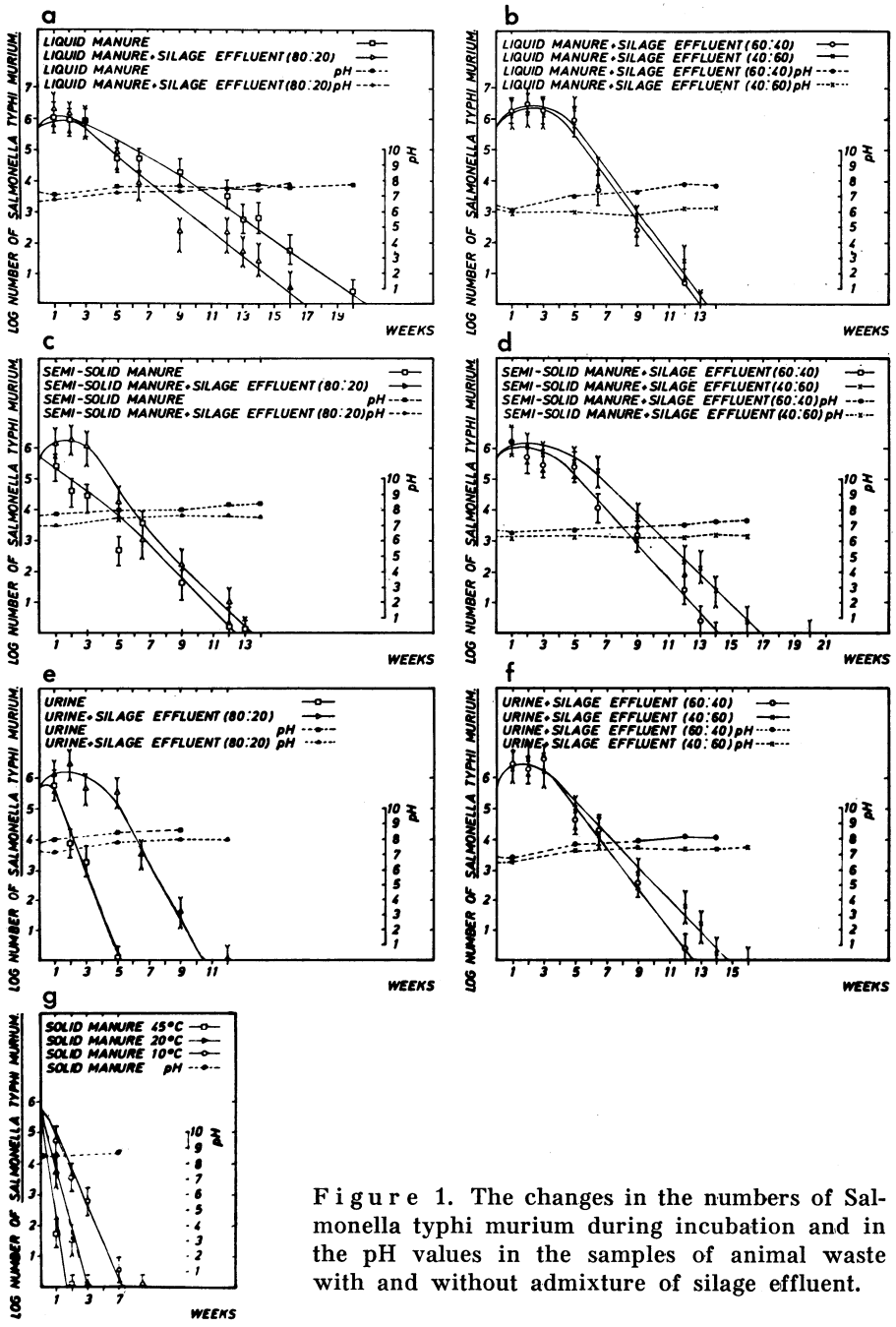

Figure 1. The changes in the numbers of Salmonella typhi murium during incubation and in the pH values in the samples of animal waste with and without admixture of silage effluent.

Table 1. The average number of bacteria in the mixtures of animal faecal waste and silage effluent.

	Incubation temperature			
	45°C	30°C	20°C	10°C
	Number of bacteria × 10^5			
Liquid manure		125	76	45
Liquid manure + silage effluent (80 % + 20 %)		188	154	118
Liquid manure + silage effluent (60 % + 40 %)		242	178	208
Liquid manure + silage effluent (40 % + 60 %)		32	26	15
Semi-solid manure		161	150	156
Semi-solid manure + silage effluent (80 % + 20 %)		247	213	197
Semi-solid manure + silage effluent (60 % + 40 %)		212	190	196
Semi-solid manure + silage effluent (40 % + 60 %)		64	40	21
Urine		12	5	2
Urine + silage effluent (80 % + 20 %)		234	223	153
Urine + silage effluent (60 % + 40 %)		401	313	188
Urine + silage effluent (40 % + 60 %)		430	358	222
Solid manure	3000	320	21	0.4

The results given in Table 1 are the arithmetical averages of 4 enumerations. Increasing additions of silage effluent to the original materials (liquid manure, semi-solid manure and urine) generally gave higher numbers of bacteria surviving. However, in the samples of liquid and semi-solid manure with the highest concentration of silage effluent this effect could not be demonstrated. With few exceptions the bacterial counts fell at lower incubation temperatures, but the differences between the results obtained at 30°C, 20°C and 10°C were generally small. Solid manure was an exception, showing a very high count of bacteria at 45°C and distinctly decreasing counts at the lower incubation temperatures.

The pH values slowly increased during the test period. The urine samples reached the highest pH level of all samples, pH 8.6. The increase during the storage period was of the same order in most samples, generally from 0.5 to 1.0 pH units. The addition of increasing amounts of silage effluent produced a greater decrease in the pH values in the last 2 test series, and the pH values did not generally increase in the samples with low pH.

Using the agar diffusion test, no inhibitory substances against Salmonella typhi murium or Sarcina lutea could be demonstrated

in any of the samples. In some mixtures, however, the acidity of the samples produced small zones of growth inhibition, but these were not present after adjusting the pH to 7.0.

DISCUSSION

A survival time of 20 weeks for Salmonella typhi murium in liquid manure is in fairly good accordance with the results of *Best et al.* (1971) whose average survival time for this bacteria was 25 weeks. *Rankin & Taylor* (1969) and *Tannock & Smith* (1972) showed that Salmonella typhi murium could persist for 12 and 16 weeks, respectively, in faeces suspensions.

The persistence of other Salmonella serotypes in liquid manure may be different from that of Salmonella typhi murium. The tenacity of S. dublin, S. paratyphi B and S. gallinarum in liquid manure is generally less than that of S. typhi murium, while S. enteriditis, S. cairo, S. bredeney, S. livingstone and S. eimsbuettel persisted for a longer time than S. typhi murium (*Strauch & Hahn* 1968, *Burrows & Rankin* 1970, *Best et al., Jeffrey* 1971 and *Findlay* 1972).

In the semi-solid manure the number of Salmonella typhi murium was reduced more rapidly than in liquid manure. The difference of about 8 weeks greater persistence in liquid manure was repeated in all 3 test series. The results of this work correspond to the findings of *Blum* (1968) who demonstrated that Salmonella typhi murium could survive in manure for 3, 9 and 12 weeks in different tests.

After 14 days at 45°C in the solid manure with bedding, which had an original temperature of 51°C in the manure storage, no Salmonella typhi murium could be detected, and the persistence of the Salmonella test bacteria was also low in solid manure stored at lower temperatures, 3 and 5 weeks, respectively, at 20°C and 10°C. According to *Blum,* Salmonella was killed in composted manure within 3 to 9 days.

Even though fresh urine may permit growth of Salmonella the first and second day after inoculation, urine and especially old, fermented urine, is an unfavourable medium for pathogenic bacteria (*Blum*). The findings in this study indicate survival of Salmonella typhi murium for 5 weeks in urine from a urine tank, and these observations disagree, to some extent, with the results of *Blum* who presented survival times in the range of hours to

2 weeks, varying with the storage temperature and the age of the urine. One explanation to this difference may be the unavoidable addition of faeces to the urine tank, which gives an increased buffer capacity and a higher content of dry matter.

The addition of silage effluent to the different types of original material (liquid manure, semi-solid manure and urine) produced different effects on the persistence of Salmonella typhi murium as seen in Figs. 1 a, b, c, d, e and f. The reasons for the dissimilarities between the samples are complex, and the effects of the antimicrobial factors on the persistence of pathogenic bacteria in a strange environment may be consequently difficult to predict.

It is reasonable to believe that the biological activity of the material is an important factor. In solid manure, the high content of thermophilic bacteria and the high temperature of the manure at sampling indicate the possibility of microbial activity which may have some sort of antimicrobial effect on pathogenic bacteria. However, an inhibitory effect against Salmonella typhi murium or Sarcina lutea could not be demonstrated by the agar diffusion test neither in the composted manure nor in any of the other samples. The microflora of silage effluent, which is dominated by lactobacilli (*Pedersen et al.* 1973), may have an antagonistic effect on the pathogenic bacteria, and this may explain the reduced survival times in mixtures of liquid manure and silage effluent.

The addition of silage effluent caused a decrease in the pH values of the mixtures. The effect of this decrease is dependent on the quantity of the addition, the buffering capacity and the original pH of the material. The changes in the pH values during the test period are similar to the observations of *Strauch & Hahn*. The pH values at the beginning of the period were, however, generally higher in their experiments and the decreases in the pH during the first weeks were more marked.

Although Salmonella typhi murium is generally resistant to changes in acidity or alkalinity, the high pH values of urine are of importance for the reduction in numbers of this test bacteria. The same effect may be demonstrated in the acid mixture of manure and silage effluent (40:60) in the 2 last experiments, where the pH decrease and corresponding inhibitory effect of the acidity were more marked than in the experiment presented in Figs. 1 b and d. However, the relatively small changes in pH

values, and the relatively large differences in survival times for urine and semi-solid manure indicate the effects of antimicrobial mechanisms other than the pH.

The moisture content of the mixtures is also an important factor for microbial activity. The low persistence of Salmonella typhi murium, even at low temperatures, in solid manure with a content of dry matter of 25.5 % may be due, to some extent, to the low humidity permitting sufficient aeration of the manure. In the samples with semi-solid manure and silage effluent the increased moisture content of the mixtures may contribute to the increased persistence corresponding to the addition of silage effluent.

The temperature of manure with low biological activity will adjust itself to the environmental temperature. *Strauch & Hahn* demonstrated that the persistence of some Salmonella serotypes is greater at 8°C than at 17°C. The results of the experiments with solid manure in this work agree with those findings.

The figures showing the counts of Salmonella typhi murium, indicate a small initial multiplication of the bacteria. This increase in the number of bacteria may be a consequence of the experimental conditions as the inoculated bacteria were harvested from a broth culture in active growth, and a duplication, or multiplication, of bacteria already committed to division may have taken place after inoculation into the new environment. This type of multiplication has been demonstrated in quantitative examinations by *Blum,* but not by *Rankin & Taylor.*

In these experiments, the samples were seeded with Salmonella typhi murium at a concentration of 5×10^5 bacteria per ml. In the faeces of cows with salmonellosis the number of bacteria excreted may vary within wide limits, according to *Findlay* (1972) up to 15×10^5 bacteria per g wet faeces. In manure with lower concentrations than in these experiments the final demonstration of Salmonella typhi murium will vary according to the decimation time (time for 1 log decrease). Assuming that the decline in bacterial counts shown in the figures is linear, the following decimation times may be indicated: liquid manure, 3 weeks; liquid manure with silage effluent (40:60), 1.5 weeks; semi-solid manure, 2 weeks; urine, 6 days; solid manure (10°C), 6 days and (45°C) 2 days.

CONCLUSIONS

1. Addition of silage effluent to liquid manure produces a mixture which, compared to liquid manure, gives reduced persistence of Salmonella typhi murium. However, the survival times of Salmonella typhi murium are still too long for a general recommendation of this method of storage of silage effluent.
2. The addition of silage effluent to urine or to semi-solid manure is not recommendable.
3. The manure should be removed before the manure tank is used for the storage of silage effluent.
4. From an epidemiological point of view, the procedures for handling manure should aim at a solid manure which may reduce the survival of microorganisms.

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REFERENCES

Best, E., W. Müller & D. Strauch: Untersuchungen über die Tenazität von Krankheitserregern in tierischen Fäkalien. 4. Mitteilung: Tenazitätsversuche mit Salmonellen in natürlich gelagerten Flüssigmisten von Rindern und Kälbern. (Studies on the viability of pathogens in animal faecal wastes. 4. The tenacity of salmonellae in liquid and semi-liquid manures of cattle and calves stored under natural conditions). Berl. Münch. tierärztl. Wschr. 1971, *84*, 184—188.

Blum, J.: Untersuchungen über Vorkommen, Tenazität, Wachstum und Desinfektion von Salmonellen in Abwasser von Landwirtschaftsbetrieben. (Occurrence, survival, growth and disinfection of salmonella in farm effluents). Schweiz. Arch. Tierheilk. 1968, *110*, 243—261.

Burrows, M. R. & J. D. Rankin: A further examination of the survival of pathogenic bacteria in cattle slurry. Brit. vet. J. 1970, *126*, 32—34.

Findlay, C. R.: The persistence of Salmonella dublin in slurry in tanks and on pasture. Vet. Rec. 1972, *91*, 233—235.

Jack, E. J. & P. T. Hepper: An outbreak of Salmonella typhi murium infection in cattle associated with the spreading of slurry. Vet. Rec. 1969, *84*, 196—199.

Jeffrey, D. C.: Persistence of three Salmonella spp. in bovine faeces. Vet. Rec. 1971, *88*, 329.

Lov om vern mot vannforurensning av 26. juni 1970. (Water Pollution Act of June 26, 1970).

McCrady, M. H.: The numerical interpretation of fermentation — tube results. Infect. Dis. 1915, *17,* 183—212.

Pedersen, T. A., R. A. Olsen & D. M. Guttormsen: Numbers and types of microorganisms in silage and effluent from grass ensiled with different additives. Acta agric. scand. 1973, *23,* 109—120.

Rankin, J. D. & R. J. Taylor: A study of some disease hazards which could be associated with the system of applying cattle slurry to pasture. Vet. Rec. 1969, *85,* 578—581.

Strauch, D. & G. Hahn: Untersuchungen über die Tenazität von Krankheitserregern in tierischen Fäkalien. 1. Mitteillung: Die Tenazität von Salmonellen in Flüssigmisten. (Studies on the viability of pathogens in animal faecal wastes. 1. The viability of salmonellae in liquid manure). Berl. Münch. tierärztl. Wschr. 1968, *81,* 441—444.

Tannock, G. W. & J. M. B. Smith: Studies on the survival of Salmonella typhimurium and Salmonella bovis morbificans on soil and sheep faeces. Res. vet. Sci. 1972, *13,* 150—153.

SAMMENDRAG

Overlevelse av Salmonella typhi murium i forskjellige typer gjødsel med og uten tilsetning av silopress-saft.

Salmonella typhi murium NVH 550 i en konsentrasjon av 5×10^5 bakterier pr. gram ble påvist i blaut og halvfast gjødsel henholdsvis 20 og 12 uker etter podning av bakteriene. I urin og fast gjødsel som også ble oppbevart ved 10 °C, overlevde testbakteriene ca. 5 uker. Salmonella typhi murium lot seg ikke påvise i fast gjødsel oppbevart ved 45 °C i 14 dager.

Tilsetning av silopress-saft til endelige konsentrasjoner på 20, 40 og 60 % reduserte overlevelsestida i blandinger med blaut gjødsel, mens en påviste den motsatte virkning i blandinger med silopress-saft og halvfast gjødsel eller urin.

Hemmende stoffer overfor Salmonella typhi murium eller Sarcina lutea NVH 546 ble ikke påvist i noen av blandingene ved agardiffusjonsmetoden.

Virkningen av miljøfaktorer på overlevelsesevnen av Salmonella typhi murium i ulike gjødseltyper er diskutert og satt i relasjon til de påviste forskjellene i overlevelsesevne.

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