Interim Report R&D Project 434

Pathogens from Farming: Fate and Transport in Soil. Interim Report, January 1994

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434/6/SW

CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

This Interim Report has been produced as part of the R&D Contract Nº 434, 'Pathogens from Farming: Fate and Transport in Soil', the overall objective of which is to study the incidence of pathogenic micro-organisms in soils receiving livestock wastes, and to investigate the persistence and mechanisms of transport of these organisms in the soil. The project has been divided into three stages, Stage I being a review of current understanding, Stage II being laboratory-based studies, and Stage III being field-based studies. This report is produced as part of Stage II, the specific objectives of which are to use laboratory-based systems to study the decay of pathogens in slurries, and to use model systems (soil cores) to study the persistence and transport of pathogens in soils.

Originally this report was intended to make a case for proceeding to Stage III of the work, which would involve validating the findings of Stage II under field conditions. However, the work during Stage II has not progressed as well as had been hoped, and following discussions with the NRA Project Leader for the contract, it has been agreed that it is not yet appropriate to proceed to Stage III, and that more work is needed in Stage II. Therefore this report summarises the progress so far and the difficulties which have been met, and indicates what further studies are needed.

Studies of the decay of pathogenic organisms in stored slurries have been successful, and indicate that the survival is inversely related to temperature. Times for 90% reductions in numbers (T_{90}) for the hardier organisms such as *Salmonella* ranged from 73 days at 8 °C to 9 days at 22 °C. Results for *Campylobacter*, which is less stable, ranged from T_{90} values of 24 days at 8 °C to 6 days at 22 °C. This shows that pathogens have the potential to persist in slurries for considerable periods, but the significance of this depends on the general occurrence of pathogens in slurries, and on the normal temperatures of storage.

Studies on transport of pathogens through soil cores have not been successful. Some reasons for this have been identified, and more experiments are to be carried out using different experimental procedures. Work is under way to carry out a wide survey of the occurrence of pathogens in livestock wastes, by having samples from different geographical areas sent to the WRc laboratories for screening.

KEY WORDS

Farm waste, bacteria (faecal), pathogenic organisms, soil.

1. INTRODUCTION

This Interim Report has been produced as part of the R&D Contract Nº 434, 'Pathogens from Farming: Fate and Transport in Soil', the overall objective of which is to study the incidence of pathogenic micro-organisms in soils receiving livestock wastes, and to investigate the persistence and mechanisms of transport of these organisms in the soil. This project has been divided into three stages, Stage I being a review of current understanding, Stage II being laboratory-based studies, and Stage III being field-based studies. This report is produced as part of Stage II, the specific objectives of which are to use laboratory-based systems to study the decay of pathogens in slurries, and to use model systems (soil cores) to study the persistence and transport of pathogens in soils. The original intention was for the report to make a case for proceeding to Stage III of the work, which would involve validating the findings of Stage II under field conditions. However, the work during Stage II has not progressed as well as had been hoped. Delays have been caused by technical difficulties (described later), and by the original Contract Manager leaving WRc for alternative employment.

Following discussions with the NRA Project Leader for Contract 434, we feel that it is not yet appropriate to make a case for moving to Stage III, and that more work is needed in Stage II. Therefore this report will be used to summarise the progress so far and the difficulties which have been encountered, and to indicate what further studies are needed.

1.1 Background

Over the years, changes in farming practice, and in particular the trend towards intensification, have led to an increase in the potential to cause pollution. In rural areas, where discharges of sewage and industrial effluent are less significant than in urban areas, farming can be the major cause of water pollution. In a report on water pollution incidents during 1990, the NRA (1992b) showed that farming was responsible for 36% of all major water pollution incidents. The majority of these incidents were caused by poor management of livestock waste systems, including inadequate storage capacity, and collapse of storage structures. As well as these acute incidents, there is concern over the more chronic and diffuse problems arising from farming, and in particular from disposal of the livestock wastes to land. These incidents often go unreported.

While the main concern regarding the polluting potential of livestock wastes is increase in nutrient levels in surface waters, there is also concern that pathogenic micro-organisms from agricultural sources may enter recreational waters and potable water supplies. Both the Royal Commission for Environmental Pollution (1992) and the NRA (1992a) have identified as a priority for research the possible link between pathogens in surface waters and the storage and use of livestock wastes.

The amount of waste produced by farm livestock varies widely depending on species, feed composition and water intake. When animals are grazing, any waste produced will fall direct to the land. However, it is usual for most livestock animals to be housed for at least part of the year. The waste produced is collected, stored and spread onto land at a later date. The type and volume of waste produced from housed animals is therefore

dependent upon not only the amount of dung and urine produced but also on the type of bedding and management system used.

Current practice on dairy farms is to handle waste either as a solid manure where animals are housed on straw bedding, or as a liquid slurry. Slurry can be defined as excreta, possibly mixed with bedding, rainwater or yard washings, which is liquid enough to allow it to be pumped or discharged by gravity. It is estimated that 70% of all dairy farms produce some slurry and that 40% produce mostly solids. Allowing for the fact that most young beef stock are bedded on straw, an overall production of around 50% slurry and 50% manure is likely. These are collected, normally on a daily basis, and spread onto land untreated usually after a period of storage.

A high proportion of pigs are housed in sties with minimal bedding. Most pig farm waste therefore tends to be quite dilute and is applied in a similar way to cattle slurry, although unlike cattle slurries, some pig farm wastes are treated by aerobic or anaerobic digestion before being spread on land.

Most chickens are also housed, but poultry waste tends to have a much higher dry matter content and is applied to land in a similar way to cattle manures.

Application of livestock wastes to land can be an effective method of treating and using these resources. However, a common problem is that waste storage facilities have a limited capacity and that large volumes of waste are applied to small areas of land. Recommendations on the land area required to dispose of the waste have been provided by MAFF (1991), but even with the best management practices for land disposal, large rainfall or irrigation events can flush the soil profile and produce high pollutant levels.

The use of these disposal practices, with particular regard to pathogenic micro-organisms, has been reviewed in an earlier report produced under this contract (King-Spooner *et al.* 1993). This Interim Report gives results obtained from practical experiments to examine the fate of bacterial pathogens during storage of slurries, and during application of slurries to soil. The intention was to obtain an idea of the survival of such organisms, and of their potential to pollute watercourses.

2. MICROBIOLOGICAL METHODS

This section summarises the analytical methods used to enumerate the various pathogenic micro-organisms used in the study. Standard methods were used where they were available, though the techniques were modified to suit the expected cell densities. Before analysis, slurry samples were weighed, placed in a suitable volume of diluent, dispersed thoroughly, then diluted as necessary.

As the natural occurrence of pathogenic micro-organisms in animal slurries will normally be unpredictable, pure cultures of pathogens of interest were used as inocula in the studies. The pathogens were selected on the basis of their relevance to real situations, their public health significance, and the practicality of detecting and enumerating them in a dense organic matrix containing a mixed population of microbes. On this basis the following strains were used: *Salmonella nottingham*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Escherichia coli* serotype O157:H7. The natural populations of faecal coliform organisms in the slurries were also monitored. The methods used are described in the following sections.

2.1 Faecal coliforms

Faecal (thermotolerant) coliform organisms were tested by membrane filtration using Membrane Lauryl Sulphate Medium, as described in Report 71 (DoE 1983) and the NRA Manual of Standard Methods for Microbiological Analysis (NRA 1992c). Cultures were incubated for 4 hours at 30 °C followed by 14 hours at 44 °C. Yellow (acid-producing) colonies were counted as faecal coliforms.

2.2 *Salmonella*

Water samples were tested using the procedure described in the NRA Microbiological Methods Manual (NRA 1992c). After concentration by filtration using a diatomaceous filter aid, the samples were enriched in buffered peptone water, followed by selective enrichment in Rappaport-Vassiliadis medium. Portions were then plated on XLD agar, and presumptive colonies subjected to further confirmatory tests. Spiked slurry samples were tested by direct plating on XLD medium without enrichment, and incubated for 24 hours at 37 °C. This allowed rapid quantitative results to be obtained by counting typical *Salmonella* colonies.

2.3 *Yersinia*

Samples were tested by direct plating on *Yersinia* Selective Agar (Oxoid CM653) with incubation for 24 hours at 32 °C. On this medium typical *Yersinia* colonies have a dark red centre, surrounded by a transparent border, while most other organisms produce colonies with diffuse pinkish centres and opaque outer zones (Oxoid 1990).

2.4 *Campylobacter*

Samples of water were tested by a Most Probable Number (MPN) method using Preston Enrichment Broth and CCDA (charcoal cefoperazone deoxycholate amphotericin) Selective Agar, as described in the NRA Microbiological Methods Manual (NRA 1992c). However, slurry samples were diluted and tested by direct plating on CCDA Agar. In both cases cultures on CCDA Agar were incubated for 48 hours at 42 °C in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen.

2.5 *Escherichia coli* serotype O157:H7

This organism was enumerated by direct plating on Sorbitol MacConkey Agar (Oxoid CM813) using a spread plating technique for diluted slurries, or membrane filtration for water samples. This pathogenic serotype of *E. coli* does not ferment sorbitol, and so produces colourless colonies on this medium, whereas other serotypes and other coliform organisms ferment the carbohydrate and form opaque pink colonies (Oxoid 1990). Plates were incubated for 18-24 hours at 37 °C. Unlike most strains of *E. coli*, serotype O157:H7 does not grow well at 44 °C.

3. DECAY OF PATHOGENS IN STORED SLURRIES

Volumes of cattle slurries were obtained and placed in 5-litre stainless steel containers. The pathogen cultures were grown up and enumerated, then portions were thoroughly mixed into the slurries to give suitable starting concentrations. For the first set of experiments, slurries were inoculated with *Salmonella nottingham*, *Yersinia enterocolitica*, and *Escherichia coli* O157:H7, and sampling was then carried out for a period of five weeks for these organisms, and for the natural population of faecal coliforms. The slurry mixtures were stored under different conditions, at 4 °C and 15 °C in both insulated and uninsulated containers, and at 22 °C in an uninsulated container.

In general, numbers of organisms were seen to decline with time, and in most cases a reasonable line of best fit could be drawn on plots of the logarithms of the bacterial counts against time. The results are summarised in Table 3.1, which shows the T_{90} values calculated from linear regression analysis of the logarithmically-transformed data, and the associated correlation coefficients. Results for *E. coli* O157:H7 were not obtained, as the method used suffered from excessive interference. For subsequent experiments the procedures were modified to overcome this.

Table 3.1 Summary of results from initial slurry storage tests

Storage temperature	Organism	T_{90} (days)	Correlation coefficient r
4 °C	F. Coliforms	49	0.36
	Salmonella	24	0.79
	Yersinia	20	0.81
4 °C (Ins)*	F. Coliforms	34	0.71
	Salmonella	35	0.78
	Yersinia	30	0.77
15 °C	F. Coliforms	13	0.93
	Salmonella	19	0.93
	Yersinia	15	0.91
15 °C (Ins)*	F. Coliforms	14	0.93
	Salmonella	17	0.86
	Yersinia	15	0.92
22 °C	F. Coliforms	9.8	0.86
	Salmonella	10	0.96
	Yersinia	15	0.77

* Denotes insulated container

In general survival appeared better at low temperatures, as would be expected, but aspects of the experimental methodology needed attention, and a second series of tests was carried out. Three fixed storage temperatures were used (8 °C, 15 °C and 22 °C), and mixtures were also placed in an incubator switching between 8 °C and 18 °C on a 12-hourly cycle to simulate diurnal variations. In this last case both insulated and non-insulated containers were used. Only non-insulated containers were used for the other tests, as the mixtures were maintained under thermostatted conditions.

The range of inocula was increased to include *Campylobacter jejuni*, and the experimental procedures were changed to improve the enumeration of *E. coli* O157:H7. The experiments were continued for at least five weeks in most cases, though where reductions in numbers of around four orders of magnitude were achieved, the mixtures were not sampled further. Some of the tests were continued for over ten weeks. The results are summarised in Table 3.2, which is set out in a similar way to Table 3.1. Reasonably good correlation was seen between the log-transformed counts and time, and the reduced survival at higher temperatures is clearly shown.

Faecal coliforms and Salmonellae consistently survived better than the other organisms, with T_{90} values quite close to each other. The *Yersinia* was the least stable at all except the lowest storage temperature, where the *Campylobacter* decayed fastest. The *E. coli* O157:H7 was consistently less stable than the faecal coliforms in general.

The changes in survival at different temperatures are clear and seem logical, with all the organisms surviving for longer at the lower temperatures. The tests using diurnal cycling between 8 °C and 18 °C gave results intermediate between the fixed 8 °C and 15 °C tests, and the cultures survived slightly better in the insulated container, where temperature fluctuations were smaller.

Overall the results indicate that pathogenic organisms have the potential to persist for considerable lengths of time in stored slurries, although this is markedly reduced at higher temperatures. However, the sanitary significance of this survival depends on whether slurries regularly contain such pathogens or not. It was therefore decided to obtain slurry samples from a wide variety of locations to screen for pathogenic micro-organisms. This study is now getting under way, though there have been delays, as described in Section 5.

Table 3.2 Summary of results from the second series of slurry storage tests

Storage temperature	Organism	T_{90} (days)	Correlation coefficient r
8 °C	F. Coliforms	73	0.89
	Salmonella	73	0.88
	Yersinia	36	0.98
	E. coli O157:H7	42	0.90
	Campylobacter	24	0.95
15 °C	F. Coliforms	22	0.78
	Salmonella	20	0.98
	Yersinia	11	0.92
	E. coli O157:H7	13	0.98
	Campylobacter	14	0.95
22 °C	F. Coliforms	7.9	0.96
	Salmonella	8.8	0.94
	Yersinia	5.8	0.94
	E. coli O157:H7	6.6	0.96
	Campylobacter	6.2	0.92
8/18°C	F. Coliforms	29	0.82
	Salmonella	32	0.89
	Yersinia	13	0.96
	E. coli O157:H7	22	0.84
	Campylobacter	15	0.97
8/18°C (Ins)*	F. Coliforms	22	0.94
	Salmonella	24	0.95
	Yersinia	12	0.99
	E. coli O157:H7	15	0.94
	Campylobacter	19	0.93

* Denotes insulated container

4. PERCOLATION EXPERIMENTS USING SOIL CORES

Soil percolation experiments were carried out using lysimeters based at Silsoe College, Bedfordshire. The lysimeters were in the form of core containers buried in the ground, with arrangements to collect any water which had percolated through the soil. Ten lysimeters were used, each containing a core of 0.9 m diameter and 1.5 m depth. The cores themselves were of re-packed soil, typical of that in the immediate locality. This was mainly sand, with a total porosity of about 40%. The cores had been in place for about seven years, and were considered to have a soil profile similar to that found *in situ*. The surfaces of the cores were grassed, and the lysimeters were covered and allowed to dry before the experimental programme was started.

The slurry applied to the surface of the cores was spiked with the following organisms, to give a cell density (in organisms per gram wet weight) as shown:

Salmonella nottingham; 1.4×10^{6} cfu.g^{-1}
Campylobacter jejuni; 1.7×10^{4} cfu.g^{-1}
Yersinia enterocolitica; 2.0×10^{4} cfu.g^{-1}

Slurry was dosed on the surface of the cores at the rate of 50 m^3 per hectare, as recommended in the Code of Good Agricultural Practice (MAFF 1991). This equated to 3.2 litres per core. Samples of percolate and of the applied slurry were taken over a period of three to four weeks, and analysed for the organisms shown above, and also for faecal coliform bacteria (natural population). The volume of percolate was also measured.

Table 4.1 summarises the results of tests on samples of the applied slurry taken from the surface of the cores. The figures shown are the geometric means of the results obtained from ten cores, and the mean dry matter in the slurries on each sampling day.

Table 4.1 Geometric mean counts of organisms in slurries applied to soil cores

		Geometric mean organisms per g wet weight			
Day Nº	% Dry matter	FC	*Campylobacter*	*Salmonella*	*Yersinia*
0	7.5	8.7×10^{5}	9.1×10^{4}	2.7×10^{5}	1.6×10^{5}
1	14.2	5.1×10^{5}	3.0×10^{4}	2.6×10^{5}	8.1×10^{4}
4	19.5	-	6.1×10^{4}	1.4×10^{5}	1.2×10^{5}
7	29.6	2.6×10^{4}	3.6×10^{5}	7.7×10^{3}	-
20	24.0	9.8×10^{3}	$<1.0\times 10^{4}$	5.7×10^{3}	-

The results indicate a general decline in numbers with time, although there is considerable variation. However, this could be partly caused by the difficulty of sampling such

material. The volumes of percolate collected were small, and none of the applied organisms were detected in any of the percolate samples. With hindsight it seems that the reliance on natural rainfall was misguided, and that some form of artificial irrigation would be much more suitable. Also it would seem advisable to run the experiments for a much longer period, and to aim to dissect the cores at the end to find whether organisms are trapped within the soil column.

5. DISCUSSION

The percolation experiments carried out to date have clearly been unsatisfactory, and work is under way to establish a better experimental design and sampling regime. It is intended to examine more types of soil, including clays and loams as well as sandy soils, as the type and structure of the soil is likely to have a major affect on percolation rates and binding of organisms to particles. Smaller cores will be used, though these are more prone to edge effects, but this will be addressed in the work. At the moment the most likely format is a core of about 150 mm diameter and 1 metre length, obtained by driving a plastic liner fitted with a metal cutter into the soil, and excavating around to remove the core. It is acknowledged that this does not concur with Section 7(d) of the Project Investment Appraisal, where cores of one metre diameter are specified. However, experience with the lysimeters at Silsoe College has shown that this scale of operation has practical difficulties, and following discussions with the NRA Project Leader it now seems more sensible to use smaller-scale cores so that a wider range of experimental treatments can be covered.

The experiments will be carried on for longer than the first set. Different starting saturations will be used, and different rates of irrigation will be applied to replicate cores. Natural rainfall obviously cannot be relied upon, so it is intended to site the cores in a large greenhouse at the WRc Medmenham site, and to apply irrigation water artificially in metered quantities. At the end of the experiments it will be possible to dissect the cores and to test for survival of organisms within the soil and for migration through the column.

Tests to measure the distribution of pathogens in animal wastes have been delayed. The intention was to arrange for slurry samples to be sent to WRc for testing from various parts of the UK. Contacts were made with representatives of SNIFFER in Scotland, but so far replies concerning the arrangements have not been received. To speed up the process, we are currently contacting farmers within easy reach of the Medmenham Laboratory, to arrange for collection of samples from various types of livestock. A number of potential contacts within the NRA have also been identified by the Project Leader.

REFERENCES

DoE (1983) *The Bacteriological Examination of Water Supplies 1982*. Reports on Public Health and Medical Subjects Nº 71. London: HMSO.

King-Spooner, C.V., Bryan, J.J. and Rees, Y. (1993) *Pathogens from Farming: Fate and Transport in Soil - A Review*. R&D Note Nº 168. Bristol: National Rivers Authority.

MAFF (1991) *Code of Good Agricultural Practice for the Protection of Water*. Ministry of Agriculture, Fisheries and Food, Welsh Office Agriculture Department.

NRA (1992a) *The Influence of Agriculture on the Quality of Natural Waters in England and Wales: A Report of the National Rivers Authority*. Water Quality Series Nº 6. Bristol: National Rivers Authority.

NRA (1992b) *Water Pollution Incidents in England and Wales - 1990: A Report of the National Rivers Authority*. Water Quality Series Nº 7. Bristol: National Rivers Authority.

NRA (1992c) *Manual of Standard Methods for Microbiological Analysis*. R&D Report Nº 2. Bristol: National Rivers Authority.

Oxoid (1990) *The Oxoid Manual, 6th Edition*, edited by E.Y Bridson. Basingstoke: Unipath Ltd.

Royal Commission on Environmental Pollution (1992) *Sixteenth report. Freshwater quality*. London: HMSO.