DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS **CSG 15**

Research and Development

Final Project Report

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Project title	**INVESTIGATION OF THE ROUTES BY WHICH PATHOGENS ASSOCIATED WITH LIVESTOCK SLURRIES AND MANURE MAY BE TRANSFERRED FROM THE FARM TO THE WIDER ENVIRONMENT**
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Executive summary (maximum 2 sides A4)

Livestock manures are a valuable source of crop available nutrients, but can also contain pathogenic micro-organisms which may reach the water and air environments. In order to manage these risks, it is necessary to assess pathogen sources and potential loss pathways, so that practical advice on reducing the risks of transfer can be developed.

An initial desk-study reviewed data on the movement of pathogens from animal housing, manure storage systems and following landspreading and grazing, to the water and air environments. Experimental work then sought to examine the routes and processes involved in the transmission of pathogens during and following landspreading, and to quantify losses of viable pathogens to the water and air environments. A non-pathogenic strain of *E. coli* was used as a marker to track both movement to water and via aerial dispersion.

Transport to water: Work on pathogen transport to water was carried out using hydrologically isolated plots, where the aim was to study 'worst-case' risk situations following the landspreading of manures and during cattle grazing i.e. on heavy-textured underdrained soils, where 'by-pass' flow was likely to provide a rapid and important loss route. Also, small plots and soil monoliths were used to examine in more detail the effects of soil type on pathogen transfer routes to surface and groundwaters.

The behaviour in slurry of the *E. coli* marker organism was similar to that of generic *E. coli*, however, the marker was less robust than generic *E. coli* when introduced into the soil environment. The marker organism was detected in drainage and surface waters where rainfall events generated drainflow/runoff a few days after manure application, but not where drainage occurred after a longer time period (i.e. weeks). This indicated that the marker was not able to survive for long periods in the soil and was only suitable for use as a short-term marker of pathogen movement (most of the field experiments used the K12 marker organism).

Drainage waters from fields which were being grazed by cattle or where manures had been recently applied contained *E. coli* concentrations which exceeded the EC limit for bathing water quality (2000 colony forming units-cfu/100ml). Also, *E. coli* was detected in surface runoff samples collected during grazing and following recent manure application.

The soil monolith experiments indicated that there was little vertical movement of slurry-borne pathogens in free draining soils, where rainfall volumes immediately after slurry application were low.

Transport to air: The transmission of pathogens via aerosol dispersion downwind of a landspreading source was studied by collecting aerosol particles on agar plates, using specialist portable air sampling units. Some short-range dispersion modelling was also undertaken. The measurements showed that aerosol-borne pathogens generated during the landspreading of cattle slurry and dirty water using a splash-plate applicator, could travel at least 200m at wind speeds of 2 m/s and over 400m at wind speeds of 4.2 m/s. Projections from these data indicated that some pathogens could be transported at least 1500m during slurry spreading.

Risks of pathogen transfer: Information from the desk-study and experimental work was used to estimate the relative risks of pathogen transfer from manures/excreta to the water and air environments. The highest risks to water from the *landspreading* of manure were assessed to be direct application into watercourses and where drainflow or surface runoff occurred from 'wet' soils within 7 days of landspreading. Similarly, the highest risks to water during *grazing* were assessed to be the direct deposition of excreta into watercourses and where drainflow or surface runoff occurred during or soon after the end of grazing. The most important *point-sources* identified were uncontained runoff from farmstead hardstanding areas and woodchip corrals, and runoff from 'wet' farmstead middens. The highest risks from aerosol transmission were assessed to be from slurry/dirtywater spreading using *rainguns and vacuum tankers* with splash-plates, particularly where wind speeds were high and fresh slurry was being spread. It was not possible to make absolute risk comparisons between the different loss routes, but it appears likely that the risks of transfer are considerably higher via water (particularly from farmstead hardstandings, following manure spreading and during livestock grazing) than air, and that the risks via other vectors (e.g. wild animals and flies) are relatively low.

Practical recommendations: Potential pathogen control measures to minimise the risks of pathogen transfer from livestock manure management systems to the water and air environments were reviewed. These included the manipulation of diet and dietary additions, minimum storage periods (typically 90 days) for slurry and solid manures, avoidance of recontamination of stored manure, slurry treatment, solids composting, landspreading methods and timing, management of grazing livestock in the field, management of farmstead runoff and general good practice. The effectiveness and reliability of the suggested measures to reduce microbial pathogen loads was assessed, both in terms of maximum potential reduction (from one to six $\log_{10}$ reduction) and reliability (i.e. is the process likely to be easily controllable under typical farm conditions). For handled manures, measures associated with extended storage periods or the treatment of solid manures and slurries generally gave the best reductions in pathogen loads, ranging from two to six $\log_{10}$ reductions, and represented the most practical and cost-effective options to reduce the risks of pathogen transfer during manure spreading to the water and air environments. In terms of field management, the avoidance of spreading manures directly into surface water systems, fencing of streams/watercourses and provision of bridges to prevent direct livestock access, and avoiding manure spreading and livestock grazing when soils are wet and drainage/runoff is likely, were assessed to be the most effective measures. In the case of point-source loss routes to surface water systems (e.g. from hardstandings, woodchip corrals etc.), investment in runoff minimisation (e.g. roofing of hardstandings) and collection/storage systems were likely to be the most cost-effective approaches.

Recommendations for future research: The highest priority for future research was identified as a need to improve our understanding of the processes and pathways through which pathogens are lost from farming systems to the *water environment*, particularly surface water systems, to provide robust data to inform agricultural mitigation measures to ensure compliance with the EU Bathing Water Directive. In parallel with this work, there is a need to synthesise our knowledge on pathogen loss routes from agricultural systems within a *modelling framework*, and in particular microbial transport in soils via vertical mechanisms and by-pass flow. There is also a need for further work on pathogen transport in *aerosols* (e.g. the effects of manure type, spreading technique and climatic conditions), as both the literature review and preliminary results from this project show that there are potential human infection risks from aerosols generated during the landspreading of manures.

Scientific report (maximum 20 sides A4)

1. INTRODUCTION AND POLICY RATIONALE

Livestock manures are a valuable source of crop available nutrients and organic matter. However, handled manures and excreta deposited directly by grazing livestock can also contain pathogenic micro-organisms, which have the potential to reach the water and air environments. In order to manage these risks, it is necessary to assess the sources and their potential loss pathways, so that practical advice can be developed to reduce the risks of pathogen losses from agriculture, which may impact on bathing water quality and shellfish beds etc.

2. OBJECTIVES

The overall objective of this project was to investigate the routes by which the management of livestock slurries and manures during housing, storage and landspreading may lead to pathogens being transferred from the farm to the wider environment. The detailed objectives were to:

- Quantify and prioritise all the possible pathogen loss routes from the farm to the wider environment, by drawing on the results of existing work.
- Examine the routes and processes involved in the transmission of pathogens during and following landspreading, and quantify losses of viable pathogens to the water and air environments.
- Assess the relative risks of pathogen transmission to the wider environment, the food chain and the public.
- Formulate practical and cost-effective recommendations to reduce any such risks to an acceptable level, that could be incorporated into the Codes of Good Agricultural Practice.
- Produce a prioritised set of recommendations for further research.

3. APPROACH

3.1. Desk-study

A literature search was undertaken to identify relevant information from experimental work in Europe and other temperate countries on pathogen loss routes, via water and air transport. The scope included potential pathogen loss routes from animal housing, farmstead hardstandings, manure storage systems, manure spreading in the field and grazing livestock. The different possible pathogen loss routes via water and air were quantified (where possible) and prioritised, and estimates made of the relative magnitude of loss risks from each of the different sources.

3.2. Experimental work

A non-pathogenic strain of *E. coli* was used as a marker to track microbial transport to water systems and via aerial dispersion.

Transport to water. The work on pathogen transport to water systems was carried out using hydrologically isolated plots, where the aim was to study 'worst-case' risk situations following the landspreading of manures and during cattle grazing i.e. on heavy-textured underdrained soils, where 'by-pass' flow was likely to provide a rapid and important loss route. Also, small plots and soil monoliths were used to examine in more detail the effects of soil and manure type on pathogen transfer routes to surface and groundwaters.

Transport to air. The transmission of pathogens via aerosol dispersion downwind of a landspreading source was studied by collecting aerosol particles on agar plates, using specialist portable air sampling units. Some short-range dispersion modelling was also undertaken.

3.3. Relative risks of pathogen transmission
Data from the desk-study and field experiments were drawn together, and a detailed assessment made of the relative risks of pathogen transmission to the water and air environments.

3.4. Practical recommendations
The results were reviewed to produce practical recommendations on management practices that could reduce the risks of pathogen losses to the water and air environments, which could be included in future revisions of the Defra Water and Air Codes (Anon., 1998; 1999).

3.5. Future research
On completion of the desk-study and experimental measurements, gaps in knowledge were identified and prioritised, and a set of recommendations for future research was produced.

4. DESK-STUDY

4.1. Loss routes to water
A large number of potential routes through which pathogens may be transported from soils to receiving waters were identified, although the factors that controlled these transfers were not always well understood (e.g. Hornberger *et al.*, 1992). Pathogenic micro-organisms may enter surface waters via overland flow (runoff) or through by-pass flow in soil profile cracks or artificial field drainage systems, or via leaching through the soil matrix of permeable soils to groundwater. The main driving force for dispersion from the soil environment to surface waters was rainfall, particularly where rainfall events generated high runoff volumes and 'turbid' flows.

Micro-organism transport mechanisms within soils can be divided into physical, geochemical and biological processes (Tim *et al.*, 1988). Physical processes include advection and dispersion. Geochemical processes act to delay pathogen transfer through the soil matrix by filtration, adsorption and sedimentation mechanisms. In addition, biological processes such as growth and chemotactic migration (movement in response to a nutrient gradient) may influence pathogen transfer through soils.

4.1.1. Loss routes following manure spreading
i) Overland flow pathways. Abu-Ashour and Lee (2000) showed that rainfall was the major factor driving both the vertical and horizontal movement of micro-organisms in soils. Similarly, recent work by Vinten *et al.* (2002) highlighted the importance of hydrological processes at the soil surface and the promotion of microbial transfers via the mobilisation of slurry colloids following raindrop impact. Fenlon *et al.* (2000) and Cook and Baker (2001) also showed that periods of heavy rainfall can cause significant losses of *E. coli* in both surface runoff and by-pass flow through the soil profile. Quinton *et al.* (2003) in a laboratory study showed that the soil incorporation of slurry following land spreading, compared with surface application, reduced faecal coliform losses in surface runoff, although pathogen survival in the soil was likely to have been increased through protection from UV light. Culley and Phillips (1982) reported that spring runoff gave the highest microbial concentrations regardless of the timing of manure application. In summary, overland flow can provide an efficient microbial transport route, but the impact of such losses on water quality is likely to be greatest in the short-term following landspreading due to microbial die-off in the soil environment.

ii) Soil matrix (leaching) flows. A large body of research has focused on the vertical transport of micro-organisms in leachate and similarities with colloid filtration theory. Vertical displacement of micro-organisms through the soil profile has been demonstrated in a variety of soil column experiments (e.g. Aislabie *et al.*, 2001; Gagliardi and Karns, 2000; Brush *et al.*, 1999; Wollum and Cassel, 1978). The initial moisture content of the soil was shown to be important in facilitating micro-organism movement, with continuous water films required to enable microbial transport. Gagliardi and Karns (2000) concluded that if soil pores were not

clogged, *E. coli* O157 was able to travel below the soil surface layers for periods in excess of 2 months following the land application of manure. Field-scale work examining the effects of manure application on microbial transport showed that whilst leachate collected at 90cm could contain faecal coliforms soon after application, they declined to undetectable levels within 30-60 days (Stoddard *et al.*, 1998). Tan *et al.* (1991) concluded that microbial transport rates were much more rapid in coarser textured soils with larger pore spaces compared with finer textured soils, a conclusion also reached by other authors (e.g. Huysman and Verstraete, 1993). More recently, soil adsorption characteristics were shown by Schijven *et al.* (2002) to influence the transport of micro-organisms. The most important soil components affecting the sorption of micro-organisms are clay and organic matter (Aislabie *et al.*, 2003), with greater *E. coli* sorption being reported on higher clay content soils due to the greater specific surface area (Ling *et al.*, 2002). Such adsorption, coupled with filtration, clearly has an important influence on pathogen transfer processes and rates.

The downward translocation of micro-organisms relies very much on pore size and the soil matrix system. Instances of pore clogging by micro-organisms will undoubtedly affect microbial transport, which will depend primarily on the pore size distribution of the soil matrix. Johnson *et al.* (1995), among others, suggested that colloid filtration theory provided a conceptual framework for modelling microbial movement. However, travel distances of micro-organisms based on filtration theory have been demonstrated to underestimate measured translocation lengths within the soil (Simoni *et al.*, 1998). Colloids can act as vehicles for pathogen transport, with large pores facilitating relatively easy colloid movement. However, to date, only a few studies have been published that detail direct evidence for colloid facilitated transport of micro-organisms (Kretzschmar *et al.*, 1999). Microbial transport can be modelled using detachment functions that are associated with microbial residence times within the soil. However, the soil ecosystem accommodates much complexity and heterogeneity, making the prediction of microbial movement a difficult task.

iii) Macropore (by-pass) flow. A number of studies reported in the literature have indicated that by-pass (or macropore) flow can be an important mechanism for pathogen transport through soils (e.g. Fontes *et al.*, 1991; Gannon *et al.*, 1991; Mawdsley, 1996; Harvey, 1997). These preferential pathways serve as routes of relatively rapid water flow and allow micro-organisms, along with other colloids and contaminants, to successfully by-pass the sieving and constraining matrix of soils. Although macropores often make up only a small volume of the soil body, they serve as important routes for both the vertical and lateral transfer of micro-organisms entrained in carrying water. Macropores may be formed naturally through soil faunal activity, plant root presence and soil shrinkage, or through the installation of field drainage systems. The role of field drainage systems on poorly drained soils and earthworms in providing large pore networks was highlighted in the literature as the two most important by-pass flow routes.

- *Drainflow losses*: Joy *et al.* (1998) showed that the contamination of tile drainflows after the application of liquid manure was strongly associated with the presence of flow in the tiles prior to application, with elevated concentrations recorded 5 days following slurry application. Concentrations exceeded 1000 colony forming units - cfu/100 ml when 8.6 mm of rain fell in 24 hours after application, but were as low as 1 cfu/100 ml where rainfall did not create drainflow until 40 days after application. Culley and Phillips (1982) demonstrated that faecal streptococci were capable of movement through the soil profile into field drains located at a depth of 75 cm, provided that water was present to facilitate the downward transport.

Work on drained soils in Scotland showed that heavy rainfall following cattle slurry application to a clay loam soil resulted in up to 7% of the applied *E. coli* being transported in the drainflow (Fenlon *et al.*, 2000). Vinten *et al.* (2002) showed that the transport of *E. coli* to drains was mainly associated with rainfall and drainflow between 3 to 7 days after cattle slurry application, with the first drainflow events after slurry application containing 10^5 to 10^6 cfu/100 ml.

Studies involving the application of simulated rainfall to isolated silty clay loam soil blocks treated with slurry containing Cryptosporidium oocysts showed that numbers collected in the runoff were initially high and stayed at a plateau of 10^3 /ml for up to 70 days after application (Mawdsley *et al.*, 1996).

- *Soil mesofauna:* Soils, and in particularly grassland soils, can contain considerable numbers of earthworms. Opperman *et al*. (1987) showed that the movement of cattle slurry and coliforms through soil was enhanced by the presence of earthworm created physical tunnels for transport. Moreover, earthworms along with mites and millipedes, were also suggested by Bowen and Rovira (1999) to act as vectors for microbial transport through the attachment of micro-organisms to such mesofauna.

4.1.2. Loss routes from livestock grazing

In addition to pathogen losses following the landspreading of manure, runoff during the livestock grazing season has also been demonstrated to transfer pathogens to surface waters (Fernandez-Alvarez *et al.,* 1991; Jawson *et al.,* 1982; Howell *et al.,* 1995), with microbial counts in runoff water increasing with stocking density (Gary *et al.*, 1983). Also, the effects of grazing on the microbial quality of runoff may persist for some time after animals have been removed from the grazing pasture (Jawson *et al.*, 1982). *E. coli* O157 was shown to persist on pasture for 105 days following excretion in sheep faeces (Ogden *et al.*, 2002). Stephenson and Street (1978) showed that faecal coliform counts in stream runoff reached concentrations of up to 2500 cfu/100 ml shortly after cattle were introduced and remained at high concentrations for up to 3 months after cattle were removed.

4.1.3. Losses during slurry storage

The failure or mismanagement of slurry storage structures can cause ‘point-source’ water pollution. Between 1987 and 1999 there were typically 2000 to 4000 substantiated water pollution incidents per annum in England and Wales from agriculture, with slurry storage accounting for 75 to 85% of all incidents from manure storage facilities (Environment Agency, 2000).

In addition, pathogens from slurry stores may contaminate groundwaters. A study on one unlined slurry store located on the Upper Chalk in Hampshire measured nutrient and microbial concentrations in porewaters at a depth of up to 76m, as a result of fissure (macropore) flow through the unsaturated zone beneath the slurry store (Withers *et al.*, 1998). In a more recent UK study, covering eight earth-based (unlined) slurry stores, although Cryptosporidium and *E. coli* O157 were present in many of the cattle slurry lagoons, neither organism was found in the aquifer material beneath (Gooddy *et al.*, 2000).

4.1.4. Losses from farmsteads

Studies carried out in the Irvine and Girvan catchments in the west of Scotland (Aitken *et al.*, 2001) indicated that the majority (58%) of all farms had middens, but nearly half of these had no containment facilities to prevent the discharge of effluent to watercourses. A more detailed survey undertaken on a sub-sample of 20 farms indicated that 60% had significant ‘point-source’ discharges to watercourses. The other main potential sources of microbial contamination of watercourses were runoff from poorly contained byres, self-feed silage aprons and cow tracks.

Recent survey data from England and Wales suggest that 80-90% of runoff from farmstead hardstandings on dairy farms and *c.*50% on beef farms is collected, with the remainder most likely seeping into proximate fields and ditches (Hatch *et al.,* 2004). Additionally, woodchip corrals are becoming increasingly common on livestock farms for the overwintering of cattle, and unless the leachate is collected and recycled with the farm slurry/dirty water, these facilities will also act as a ‘point-source’of pathogen losses.

4.1.5. Losses directly from livestock

The direct deposition of excreta into watercourses by animals has long been a cause for concern and controls have been proposed to reduce the risks associated with such practices (National Rivers Authority, 1992). The main reason for allowing animals to have access a watercourse is for drinking. Additionally, dairy cattle may also need to cross a stream or ford on the way to the milking parlour. The defecation of excreta directly into a water course or in close proximity, can contaminate surface water systems with large numbers of viable micro-organisms (Tiedemann *et al.*, 1987). Moreover, Nagels *et al.* (2002) who investigated microbial transport from livestock to surface water systems concluded that direct deposition of faecal matter into water courses was likely to be the most significant loss pathway, alongside 'wash-in' from surface runoff. A west of Scotland study (Aitken *et al.*, 2001) showed that grazing animals had access to watercourses on 50% of the farms, and that 13% of dairy herds in the River Irvine catchment and 60% in the Water of Girvan catchment crossed streams on a daily basis.

4.2. Losses to air

Aerosol emissions from livestock housing and during the landspreading of manure can result in pathogen dispersion, which could be a risk to human health through direct inhalation or via the contamination of surface water systems and crops. Aerosols vary in size from 0.01 microns to about 50 microns, with the larger particles more rapidly deposited due to gravity, although aerosols containing pathogens can travel large distances (i.e. several hundred metres).

Few studies have quantified microbial emissions from buildings, manure stores or in the field during spreading. However, there have been a number of studies with biosolids (sewage sludge), which can be used as a guide to the relative risks from livestock manure spreading. Aerosol emissions from biosolids were found to depend on manure type and pathogen concentration, and to increase with wind speed. In addition, the method of manure application also influenced aerosol dispersion.

4.2.1. Composting

No publications were identified that studied microbial dispersion during the composting of solid livestock manures. However, some analogies can be drawn from municipal and green waste composting studies. A composting plant with a feedstock of green waste in Belgium measured airborne concentrations of total bacteria, molds and yeasts that were 10- to 100-fold higher inside the covered composting area compared with the open air outside. Also, these organisms increased 1- to 4-fold in magnitude at the onset of mechanical activities (pre-treatment, compost turning and refining), with concentrations returning to their original levels after 2 to 5 hours (Maricou *et al.*, 1998)

4.2.2. Livestock housing

There were few references in the literature to livestock housing as a source of pathogen dispersal. Investigations on pig farms showed that the concentrations of air-borne micro-organisms about 100 m from pig farms were around 1700 cfu/m^3 in winter and 930 cfu/m^3 in spring (Platz *et al*., 1995). Investigations during a whole year at 8 different sampling places in an area with high livestock density revealed pronounced distance dependent micro-organisms concentrations and a huge seasonal influence on fungi concentrations (Hartung, 1992). experiments Recent found higher particle densities about 115 m downwind of a piggery using a Lidar detection device (Hartung *et al.*, 1998).

4.2.3. Land application of organic manures

The land application of slurry and solid manure is known to cause odour nuisance and gaseous emissions (e.g. ammonia volatilisation). From visible spreading plumes, we can also deduce that particulate material and aerosols are transported in the air, and that this occurs more in stronger winds. These may be inhaled by people and animals in the area, or drift onto edible crops and into surface waters. The majority of the literature

information is based on research from biosolids (sewage sludge) spreading and from the irrigation/rain gun application of raw sewage or dirty water. Coliforms have been detected hundreds of metres from sprinkler sources of raw sewage (Schultz, 1943; Shtarkas & Krasil'shehokov, 1970), with coliform concentrations dropping to background levels immediately after the cessation of spreading.

Animal viruses, coliphages and bacteria were identified in aerosols from wastewater irrigation on farms (Brenner *et al*., 1988). Liquid manure spreading resulted in microbial transmission 228 m downwind (measured using Anderson samplers) and 274 m downwind (measured using static impaction plates) from the spreading source (Evenden, 1972). Generally when the wind speed was below 2.2 m/s and samplers were 200 m from the source, only *c*.10% of the micro-organisms were recovered. Wind speeds of between 1.3 to 6.7 m/s were positively associated with increased microbial transmission distances.

Microbial contamination following the application of raw pig slurry using a 'snowgun' was dependent upon the distance from the spreading source (White *et al*., 2001). Bacterial concentrations upwind of the snowmaking guns were relatively low, however, downwind samplers had high faecal coliform and faecal enterococci concentrations in the plume.

The dispersion of pathogens by aerosol transmission can occur during the filling of slurry tankers and during landspreading. The size of particles produced during slurry handling is mostly less than 10 microns, which is small enough to enter the mammalian lung (May, 1966). Boutin *et al.* (1988) reported that there were few micro-organisms transferred by aerosol transmission to distances greater than 50 m during the use of a flail spreader with solid cattle manure (19% dry matter content and wind speed 1.8 m/sec). However, the manure was 8 months old and had a low microbial count. During a vacuum tanker application of fresh cattle slurry with high micro-organisms levels, concentrations declined to background levels after 200 m (wind speed 2.2 m/s). Boutin *et al.* (1988) also identified that the particle profile (50%>10-micron) remained stable over the first 100m from where pig slurry was applied using a rain gun, with a transfer distance of up to 200 m.

Data from the literature summarising distances (from source) over which micro-organisms have been shown to travel and remain viable are collated in Table A1 in Appendix I. To provide a comprehensive picture the source and sampling method used is also included. The transport distances ranged upto 650m, with the distance generally increasing with windspeed.

4.3. Other vectors

The transfer of pathogens present in manures can also occur through spillage from manure spreaders travelling on public roads or through streams. Rodents and birds can also act as vectors (Cowan, 1998; Sturdee, 1998; Hooda *et al*., 2000; Böhm & Hartung, 1994). Similarly, adult and larval stages of houseflies with access *to Crytosporidium parvum*-contaminated substrates can carry the oocysts in their digestive tracts and on their external surfaces (Graczyc, 1999). Similar observations have also been made for *E. coli* O157 (Moriya *et al.*, 1999) and *Campylobacter* (Nichols, 2005), where the focus has been on direct contamination of human food by flies. Whilst the literature mentions the potential importance of such vectors, there have been few measurements of pathogen transport via these routes under field conditions.

5. EXPERIMENTAL WORK

5.1. Use of *E. coli* marker strain

Escherichia coli K12 (resistant to nalidixic acid) or *Escherichia coli* W18 (resistant to kanamycin) were used as marker organisms to track the behaviour of 'native' manure *E. coli*, providing a unique indicator of manure micro-organisms. The organisms were cultured in the laboratory as described in Appendix II and are referred to throughout this report as resistant *E. coli* (REC).

5.2. Transport to water

5.2.1 Materials and methods

5.2.1.1 Grassland experimental studies at Rowden

Experiment design. The study was carried out on the Rowden drainage experiment at IGER North Wyke, which was established in 1982 on old unimproved grassland on poorly drained sloping land (5-10%). The soil is a non-calcareous pelostagnogley of the Hallsworth series, overlying clay shales, which is typical of large areas of permanent grassland in the south-west of England. The drained and undrained one hectare hydrologically isolated plots allowed transfers of REC and generic *E. coli* to be determined during and after cattle grazing, and following slurry spreading with farm-scale machinery.
There were 4 separate experiments, which aimed to:

i) Determine the behaviour of REC in slurry and soils compared with generic *E.coli* to assess the suitability of REC as a marker organism (spring 2003).
ii) Quantify micro-organism losses to water during cattle grazing (summer 2002).
iii) Quantify micro-organism losses to water following spring slurry spreading (spring 2003).
iv) Quantify micro-organism losses to water following autumn slurry spreading (autumn 2003).

i) The REC survival study was carried out using twenty replicate 2 x 2 m square plots in a randomised block design. Ten plots were untreated and 10 plots received slurry.
ii) For the grazing study in 2002, the following plots were used :
a) 2 undrained plots (1 weir collecting composite surface and subsurface flow at 30 cm)
b) 2 drained plots (2 weirs collecting composite surface and subsurface flow at 30 cm and drainflow at 80 cm).
iii) and iv) For the slurry spreading studies in spring 2003 (followed by livestock grazing) and autumn 2003, two untreated control plots were also used, viz:

- 1 undrained plot as (a) above
- 1 drained plot as (b) above.

Experimental treatments
i). Slurry inoculated with REC (K12) was applied at 16 m^3/ha on 29 April 2003
ii). The cattle went out to graze on 13 May 2002. Each animal was weighed and fresh dung was collected for analysis shortly after they went out onto the paddocks. Each plot was grazed at a mean intensity of *c*.2 livestock units/ha, with the animals removed on 21 October 2002.
iii). On 29 April 2003, slurry inoculated with REC (K12) was spread on the four plots (i.e. drained and undrained treated and untreated control plots) that had previously been grazed in 2002. Each treatment plot received 14–16 m^3/ha of slurry. All the plots were grazed once the slurry had been washed off leaves by rainfall shortly after application.
iv). Between 10 and 13 November 2003, dairy slurry was spread on the four plots that had previously received slurry in spring 2003. The slurry was inoculated with REC (K12) as before and each treated plot received 28–32 m^3/ha.
Soil and slurry analysis.

i). Soil sampling on the untreated control plots was carried out at two depths (0-2 and 0-7.5 cm) to obtain background microbial counts. Soil samples at 0-2 cm were then taken 1 and 2 days after slurry had been spread. Seven days after slurry application the two-depth sampling was repeated, followed by the 0-2 cm sampling after 14 and 21 days. The two-depth sampling was also repeated at 8 weeks. The slurry, which was stored separately, was also sampled at the same time as the soil, taking 10 replicates each time. All samples were analysed for *E. coli* and REC (see Appendix II for analytical methods).
ii). Before the cattle were turned out to graze in spring, a comprehensive soil sampling programme was undertaken at the end of April 2002, using a 4 x 4 grid. Soil sampling using a 6 x 6 grid was also undertaken during the grazing period on 6 evenly spread occasions. Soil samples were analysed for *E. coli* and REC as above.
iii and iv). Bulked slurry samples were taken on the day of application and analysed for *E. coli* and REC.

Drainage water collection and analysis.
ii). During the grazing season, drainage waters from the plots were collected on 11 occasions from 6 routine evenly spaced timings and following 5 rainfall events. Samples from the Rowden ditch (RD), which collects drainage water flows from the whole of the Rowden experiment, and from the downstream outlet into the River Taw (RT), were also collected at the same time. Water samples were analysed for *E. coli* and REC, as well as pH, turbidity, suspended solids (SS) and total phosphorus (TP).
iii). In spring 2003, drainflows were sampled from all of the running drains, before and after slurry spreading. On 2 May 2003, rainfall was sufficient to produce runoff from all of the plots, although there was no surface runoff on the drained plots. The water samples, together with those from RD and RT, were analysed for total coliforms and REC.
iv) In late November 2003, drainflows generated around two weeks after slurry spreading were monitored using an approximately 3-hourly sampling regime for 24-36 hours. Waters were sampled from all of the running drains, with samples also collected from RD and RT. Water samples were analysed for *E. coli* and REC, as well as turbidity.

5.2.1.2 Drainflow and surface runoff experiments at ADAS Boxworth (arable) and ADAS Rosemaund (grassland)

*Experiment design.*There were 2 sites, one at ADAS Boxworth (Cambridgeshire) on a clay textured arable soil (Hanslope series) and one at ADAS Rosemaund (Herefordshire) on a moderately well drained silty clay grassland soil (Middleton series). The Boxworth facility comprised 9 hydrologically isolated drained plots of 12 x 48m, with two lateral drains (24m apart) covered with permeable backfill, plus mole drains at a spacing of 2 m. The design was fully randomised, with 3 untreated (control) replicates and 2 replicates of each of the 3 manure treatments. At Rosemaund, surface runoff and drainage water samples were collected from nine 2 x 15m plots, which were each hydrologically isolated (to a depth of 1m). Each plot sloped uniformly at approximately 5^0 and at the lower end a metal plate inserted into the surface, enabled the collection of surface water runoff which was channeled into a collection tank. A sub-surface drain, installed at 0.75m depth along the long axis of each plot and backfilled with gravel to close to the soil surface, drained into a separate collection tank. A randomised block design was used, with 3 replicates of each treatment.

Manure application and analysis. In both winter 2003 and 2004, broiler litter, pig slurry and cattle FYM were surface applied to stubble at ADAS Boxworth, and cattle slurry and cattle FYM were surface applied to grassland at ADAS Rosemaund. The target application rate was 250 kg/ha total N (actual application rates are given in Table A2 in Appendix I). At ADAS Boxworth, the slurry was applied using a Tramspread (broadcast) applicator attached to a weighed vacuum tanker and at ADAS Rosemaund, using the ADAS purpose-built slurry plot applicator. The solid manures were applied by hand.

All the manures were enumerated for total coliforms, *E. coli, E. coli* O157, *Salmonella, Listeria* and *Campylobacter* (see methods in Appendix II). Additionally, the pig and cattle slurries were inoculated with REC (W18 at Boxworth in January 2003 and Rosemaund in February 2003; K12 at Rosemaund in November 2003 and ADAS Boxworth in February 2004) before spreading to act as a unique indicator of manure-borne pathogens. Cultures of REC in pre-measured quantities were supplied in sealed containers immediately before the experiment. The REC culture had a target concentration of 1×10^{11} cells/100 ml and the target concentration in the slurry was *c.*1×10^{7} cells/100 ml, so a dilution of approximately 1×10^{4} was required. The slurries were agitated using a pump both prior to and post inoculation. After mixing, slurry samples were collected for post-spike inoculant analysis (see Table A3).

Soil analysis. To assess the potential risks of pathogen transfer following manure spreading, each plot was soil sampled (0-7.5 cm depth) at the time of each drainflow event. Samples were enumerated for *E.coli*, REC (slurry plots only) and if present in the original manure, *Salmonella, Listeria, E. coli* O157 or *Campylobacter* (Table A4). Cores were taken from 25 locations per plot and bulked to give a single sample from each replicate plot. The soil corer was disinfected between each plot with a disinfectant wipe.

Drainage water collection and analysis. Water samples were taken during runoff events using automatic sampling equipment. The aim was to obtain 3 drainage water samples per plot, during each of 3 drainage events in the 3 month period following land application. At Rosemaund, drainage water flows were induced by irrigating the plots with at least 30mm of reservoir water, using a boom applicator. The automated water samplers were controlled by a datalogger, with sampling timed on a flow-weighted basis. Water samples were collected in clean 125 ml polystyrene vials. Three sample bottles were selected for analysis from each drainflow event, from the start, middle and end of the event. Additionally at Rosemaund, surface water runoff samples were also collected if they were generated. The samples (including irrigation water) were enumerated for total coliforms, *E. coli* and REC (slurry treatments only). Where *Salmonella, Listeria, E. coli* O157 or *Campylobacter* were detected in the manures before spreading, their concentrations were also enumerated in the drainage water samples.

5.2.1.3 Small plot experiments at IGER

Experiment design. Twelve grassland lysimeter plots were established on a heavy clay loam soil at IGER North Wyke, Devon. Each plot measured 6 x 3m and was isolated using a plastic liner down to 30 cm. The plots were surrounded on the downhill sides with drainage channels dug to 30cm and backfilled with clinker, collecting into a drain-pipe which fed into a tipping bucket system. Waters draining from the plots were collected on a flow-proportional basis.

Manure applications. There were 4 treatments: dairy slurry, dirty water, dairy farmyard manure (FYM) and an untreated control, with 3 replicates of each treatment arranged in a randomised design. REC (K12) was added to the slurry, FYM and dirty water to act as a unique marker organism of manure-borne pathogens immediately before application to the plots. Slurry and dirty water were applied at a rate of 50 m^3/ha, and FYM at 50 t/ha on 12 January 2004.

Sample collection and analysis. Water samples were collected before any treatments were applied and for 12 days immediately following manure application.

5.2.1.4 Soil monoliths at IGER

Experiment design. Twelve grassland monolith lysimeters of 80 cm diameter (0.5 m^2 area) and 1.35 m depth were used in this study. Four replicates of each of 3 different soil types were arranged in a randomised block design. The soil types were: Radyr Series (well drained clay loam), Newport Series (free draining sandy soil) and Frilsham Series (free draining sandy loam over chalk).

Manure applications. Slurry inoculated with REC (K12) was applied to the top of the monoliths at a rate of 50 m^3/ha, on 16 February, 1 March and 15 March 2004.

Sample collection and analysis. Slurry samples with and without REC were collected and enumerated. The volume of drainage water over the experimental period was measured and samples taken periodically for analysis. Following the first two slurry applications, drainage waters were sampled daily for 4 days and then once the following week. After the final slurry application, drainage water samples were collected as before and then again after a fortnight. Water samples were analysed for total coliforms, *E. coli* and REC.

5.2.2 Results and discussion

5.2.2.1 Grassland experimental studies at Rowden

i) *Survival of E. coli and REC in slurry and soils*. Figures 1 and 2 demonstrate that REC (K12) was able to survive longer in the slurry (up to 60 days) than following application to the soil (<14 days). The survival pattern of REC in slurry was very similar to that of generic *E. coli* (Figure 1). However, in the soil environment, REC survival was considerably less than generic *E. coli* (Figure 2), which was most probably due to REC being a less robust organism once it was exposed to a 'harsh' nutrient poor soil environment.

Figure 1. *E. coli* (log mean) and REC survival in slurry.

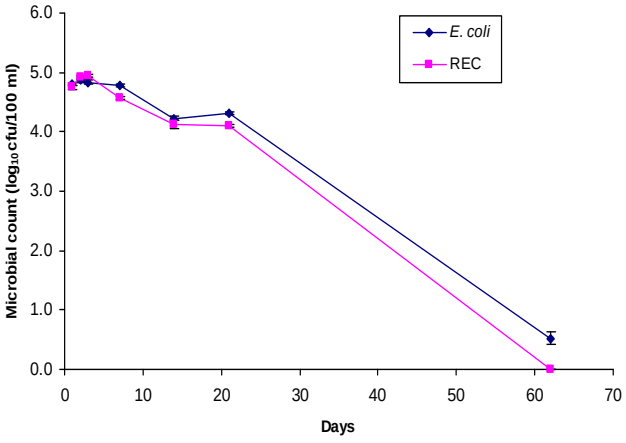
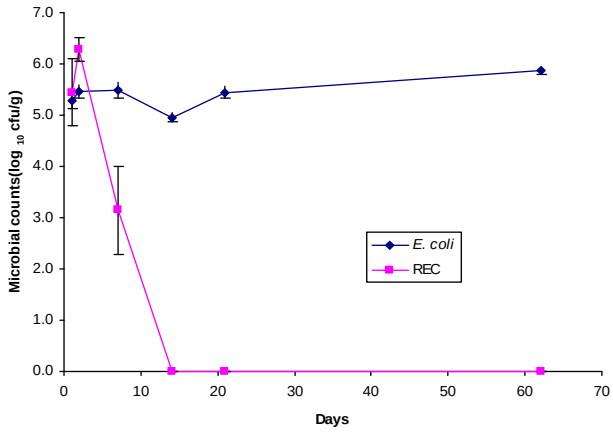
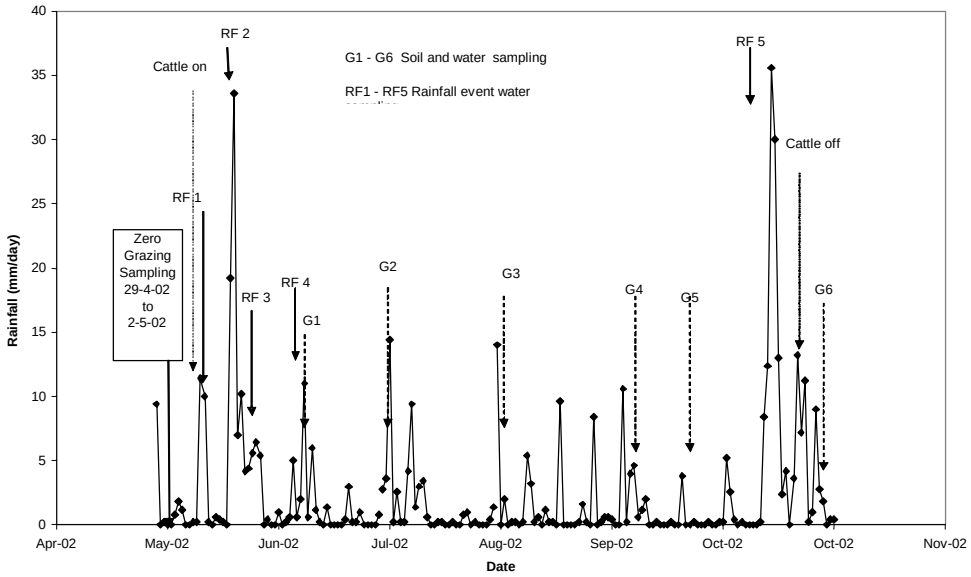


Figure 2. *E. coli* (log mean) and REC survival in soil.



ii) *Pathogen losses during grazing*. The timing of water sampling in relation to rainfall and cattle movements is shown in Figure 3. Temperatures were in the range 2 – 20°C. Manure analysis indicated that *E. coli* O157 was also present.

Figure 3. Timing of rainfall and water sampling events over the grazing period (May to October 2002).



During the grazing season, there were no clear trends in soil *E. coli* (Figure A1) or *E. coli* O157 (Figure A2) numbers, and no consistent differences between the drained and undrained treatments, with soil *E. coli* and *E. coli* O157 levels typically ranging between 0.2 and 1.8 $\log_{10}$ cfu/g soil. Drainflow volumes (Figure 4) were closely mirrored by suspended solids, turbidity and total phosphorus concentration increases (Figures A3-A5). *E. coli* concentrations in both drainflow and surface runoff waters were generally below the EC limit for bathing water quality (2000 cfu/100ml – making the assumption that faecal coliform concentrations are the same as *E.coli*. concentrations i.e. a 1:1 relationship), except from the drained plots in both drainflow and surface runoff waters after the first period of heavy rainfall in May 2002. Interestingly, the River Taw also exceeded the limit during September 2002, with concentration increases also recorded from the drained and undrained plots (Figure 5).

Figure 4. Drainage and surface runoff flows during grazing.

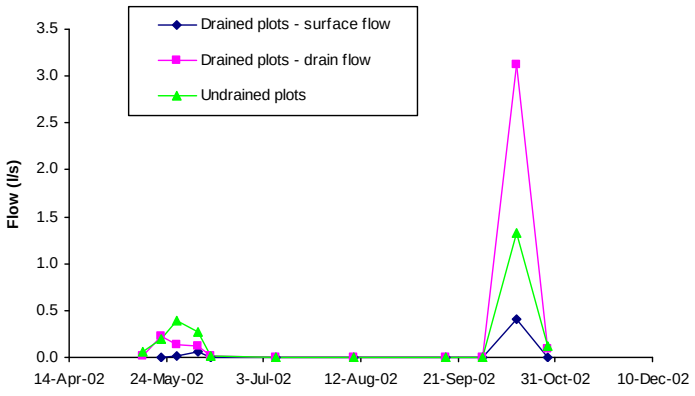
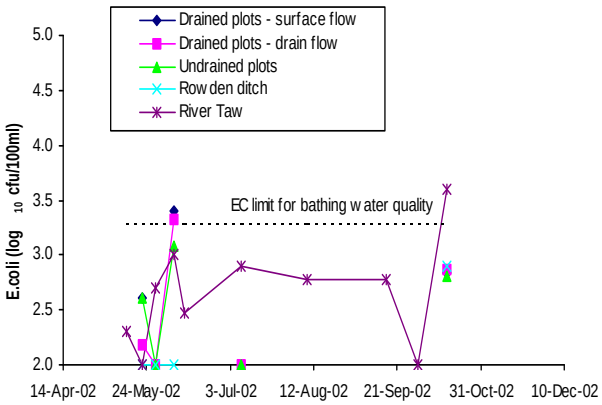


Figure 5. *E. coli* concentrations (log mean) in drainflow, surface runoff and river waters during grazing.



iii) *Pathogen losses following spring slurry spreading*. Rainfall after the spring slurry application on 29 April 2003, generated drainflow 2 days later. The presence of REC in water flows from both the drained and undrained plots, as well as in the Rowden ditch, provides unequivocal evidence that micro-organisms in slurry can reach surface waters following landspreading (Figure 6).

Figure 6. REC concentrations (log mean) in drainflow and surface runoff waters following spring slurry spreading (2003).

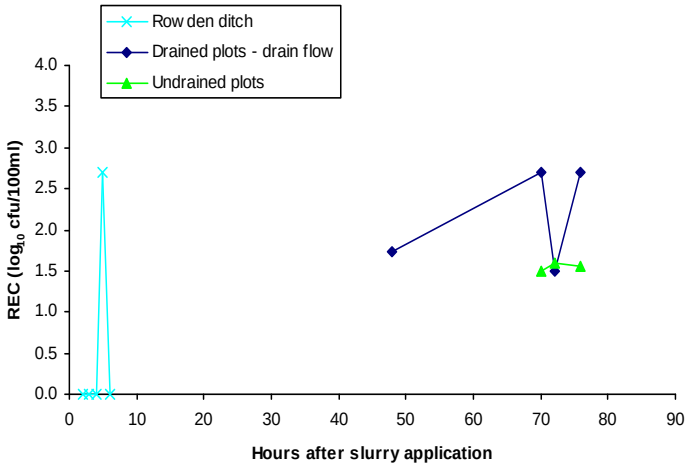
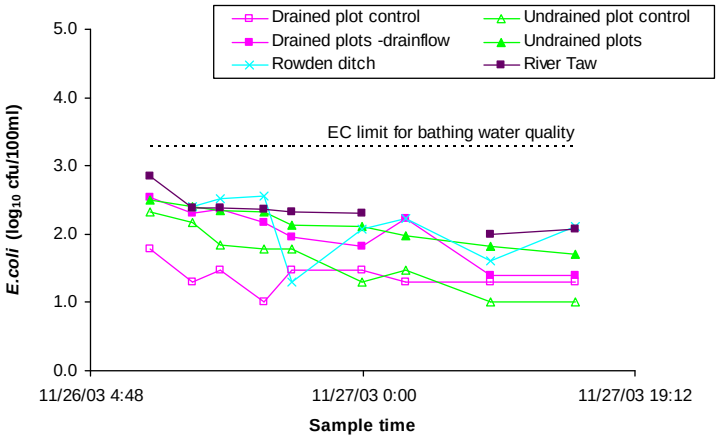


Figure 7. *E. coli* concentrations (log mean) in drainflow and surface runoff waters following autumn slurry spreading (2003).



iv) *Microbial losses following autumn slurry spreading*. REC were not detected in drainage water flows when rainfall produced drainage about two weeks after cattle slurry spreading between 10 and 13 November 2003, which was most likely due to rapid die-off in the soil environment (see Figure 2). *E. coli* concentrations in drainage water flows decreased over time through the drainage event (Figure 7). Drainage water flows from the untreated control plots (where no slurry had been applied) had lower *E. coli* concentrations than from the slurry treated plots, and the Rowden ditch and River Taw waters. All the waters had *E. coli* concentrations below the EC limit for bathing water quality (2000 cfu/100ml).

5.2.2.2 Drainflow and surface runoff experiments at ADAS Boxworth and ADAS Rosemaund

Manure analysis. Details of the manure applications are given in Table A2 and the microbiological analysis of the manures at spreading in Table A3. REC was measured in the inoculated pig and cattle slurries at around the target concentration of 10^7 cfu/100ml in both years. Listeria was detected in the pig slurry and cattle FYM spread at Boxworth in year 1, and in the cattle slurry/FYM spread at Rosemaund in year 2. No *Salmonella*, *Campylobacter* or *E. coli* O157 were detected in any of the manures.

Soil analysis. At ADAS Boxworth, total coliform and *E. coli* levels were generally higher in soils from the manured treatments than from the untreated control (Table A4). In year 2 (2004), REC were detected in soil samples collected from the pig slurry plots (on the day of spreading), although they were not detected the previous year (4 days after spreading), which was most probably due to rapid die-off in the soil environment (see Figure 2). At ADAS Rosemaund in year 1 (2003), soil samples were taken at the time of each of the three drainage events on 11 March, 8 April and 28 April 2003 (i.e. approximately 2, 6 and 9 weeks after manure application). REC were only detected in soil samples from the cattle slurry plots at the time of the first drainage event, but not thereafter.

Drainage water analysis. At ADAS Boxworth, drainage water flows occurred 4 and 1 days after manure spreading in years 1 and 2, respectively, during which 3 or 4 drainage water samples were collected from each plot (at the start, middle and end of each drainage event) for microbiological analysis. The mean total drainage volume recorded was 8mm in year 1 and 5mm in year 2. Listeria were not detected in drainflow samples in year 1, despite being present in the applied pig slurry and cattle FYM. REC were detected in drainage water flows from the pig slurry treatment, despite not being detected in the corresponding soil, demonstrating that REC can act as an effective short-term marker following slurry application to land. Although <1% of the REC applied in the slurry were recovered in the drainage water flows, REC levels were above the EC limit for *E. coli* in bathing

waters (Figures 8 and 9). *E. coli* concentrations in drainage water flows from the manured treatments were higher than from the untreated control (Figure 10). There were no consistent trends in *E. coli* concentrations during each drainage event, however, there appeared to be a trend for concentrations to decrease over time with increased drainage volumes.

At ADAS Rosemaund, there were 3 drainage events each year, with mean total drainage volumes of 11 and 28 mm in years 1 and 2, respectively. In year 1 (2003), analysis of the irrigation water used showed that total coliforms were present at 10^4 cfu/100ml, with no *E. coli* or *Listeria* detected. *E. coli* concentrations in drainage waters were higher from the manure treatments than from the untreated control, with concentrations from the FYM treatment higher than from the slurry treatment (Figures 11 and 13), which was most probably a reflection of higher *E. coli* levels in the applied FYM compared with the slurry (Table 3A). *E. coli* were also detected in surface runoff samples from the manure treatments. In year 1 (2003), where REC inoculated cattle slurry was applied, REC were only measured in water samples from the first drainflow event (at levels of 10^4 cfu/100ml), but in year 2 (2004) no REC were detected in drainflow samples. These data provide further supporting evidence to indicate that REC is not sufficiently robust to survive in the soil environment for use as a medium- to long-term marker of microbial movement. In year 1 (2003), drainage water *Listeria* concentrations were generally higher from the manure treatments than the untreated control (Figure 12).

Figure 8. REC concentrations (log mean) in drainage water flows at ADAS Boxworth - 2003 (concentration in slurry 2.2 10^7 / 100ml)

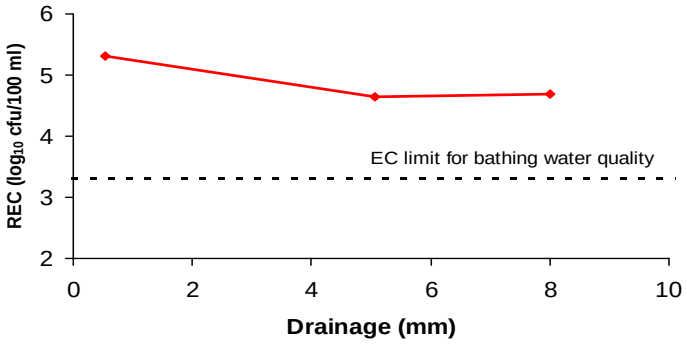


Figure 9. REC concentrations (log mean) in drainage water flows at ADAS Boxworth - 2004 (concentration in slurry 7.9 x 10^6 / 100ml)

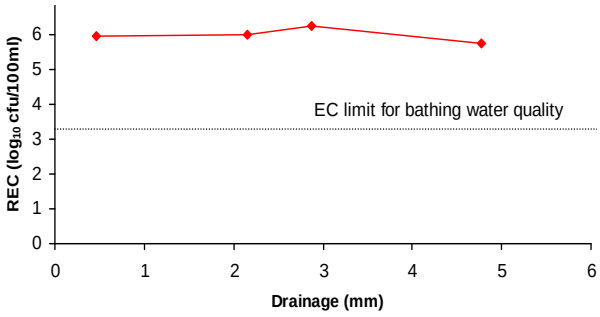


Figure 10. *E. coli* concentrations (log mean) in drainage water flows at ADAS Boxworth - 2004

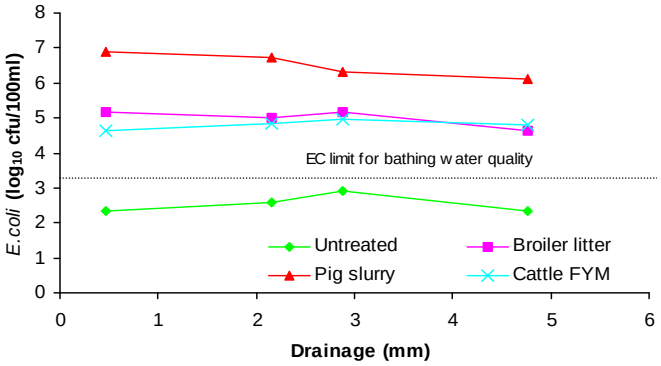


Figure 11. *E. coli* concentrations (log mean) in drainage water flows at ADAS Rosemaund - 2003

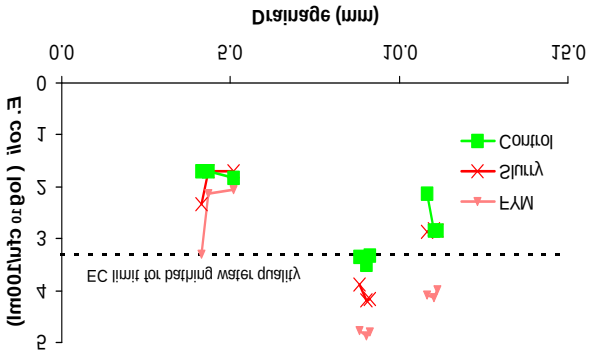


Figure 12. *Listeria* concentrations (log mean) in drainage water flows at ADAS Rosemaund -2003.

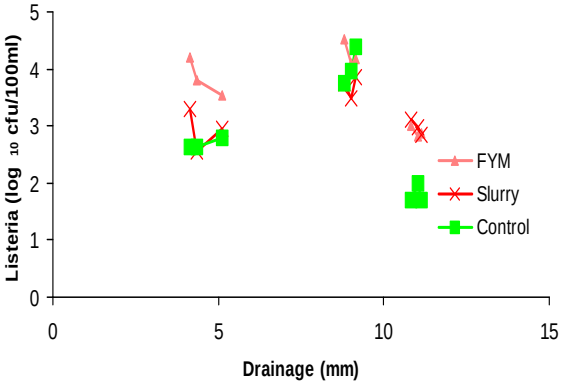
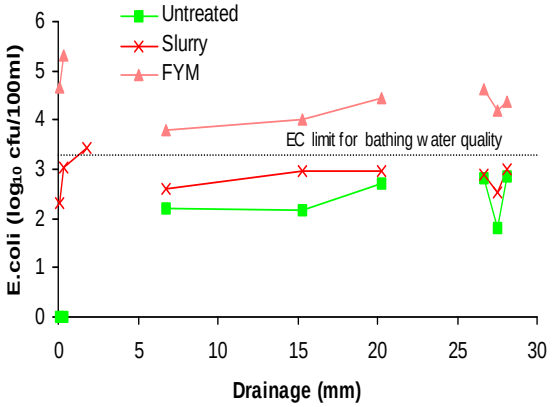


Figure 13. *E. coli* concentrations (log mean) in drainage water flows at ADAS Rosemaund -2004.



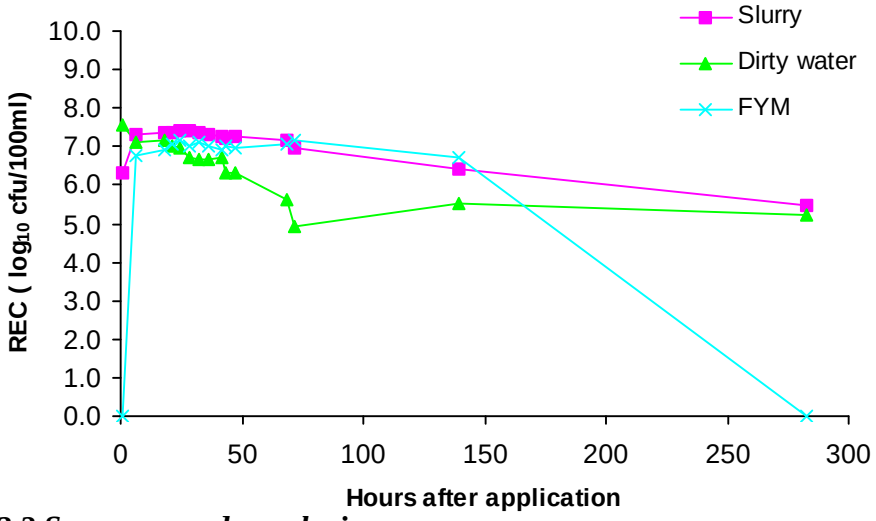
5.2.2.3 Small plot experiments at IGER

REC were detected in drainage water flows at 30 cm depth from both the slurry and dirty water treatments for up to 12 days after land application, and from the FYM treatment for up to 6 days, at concentrations which exceeded the bathing water limit of 2000 cfu/100ml (Figure 14). Also, it is noteworthy that peak REC concentrations following the slurry and dirty water applications occurred within 1 day of landspreading, whilst peak concentrations following FYM application occured between days 1 and 3.

5.2.2.4 Soil monoliths at IGER

There were no *E. coli* or REC detected in drainage water flows from any of the lysimeters after the first two slurry applications, which was probably a result of low rainfall and associated drainage volumes after application. However, following the third slurry application, when *c.*25 mm of rainfall had fallen in the 4 days after application, both *E. coli* and REC were detected in drainage waters from one of the Frilsham Series (free draining sandy soil over chalk) lysimeters at peak concentrations of >10,000 cfu/100ml. Overall, these data indicate that pathogens in slurry applied to free draining soils where water largely travels through the soil matrix, rather than via by-pass (i.e. crack) flow, are unlikely to undergo significant vertical transport in the short-term following landspreading.

Figure 14. REC concentrations (log mean) in drainage waters from small plot lysimeters at IGER.



5.2.3 Summary and conclusions

The survival of the REC marker organism in slurry was similar to that of generic *E. coli*. However, REC was considerably less robust than generic *E. coli* when introduced into the soil environment. REC were detected in drainage and surface water samples where rainfall generated drainflow and surface runoff within a few days of manure application, but not where drainflow and runoff occurred after a longer time period (i.e. weeks). Clearly, the REC was not able to survive for long periods in the soil and is only suitable for use as a short-term marker of microbial movement. Nevertheless, the presence of REC in drainflow and surface runoff waters provides unequivocal evidence that micro-organisms in slurry can enter surface water sytems following land application.

Drainage water samples collected from land that was being grazed or where manures had recently been applied, contained *E. coli* concentrations that exceeded the EC limit for bathing water quality (2000 cfu/100ml). Similarly, *E. coli* were measured in surface runoff samples collected during grazing and following the land spreading of manure.

The soil monolith experiments indicated that there was likely, in the short-term following landspreading, to be little vertical movement of slurry borne micro-organisms in free draining soils where water movement was largely through the soil matrix, rather than via by-pass (crack) flow. Although, there may be circumstances (e.g. heavy rainfall immediately after slurry application) that can result in some downward transport of micro-organisms and contamination of groundwaters.

5.3. Transport to air

5.3.1 Introduction

In this study, the transmission of pathogens via aerosol dispersion downwind of a landspreading source was measured, using specialised sampling equipment. Aerosol particles were collected directly on agar plates (with an appropriate medium according to the target organism), using portable air sampling units (Burkard samplers).

5.3.2 Materials and methods

Experiment 1. Pig slurry inoculated with REC (K12) at a concentration of 1.6 x 10^6 cfu/100 ml was pumped from a lagoon and applied using a raingun on a farm in Suffolk in April 2002. Four Burkard air samplers were located at distances of both 50 and 125m downwind from the source. The samplers were run for 1, 5 and 9 minutes to determine the optimum exposure time for the agar plates, and to establish whether REC could be recovered using this technique.
Experiment 2. Dairy slurry or dirty water were spread using a slurry tanker with a splash plate attachment at IGER North Wyke. Four runs were conducted, with REC (K12) introduced into the slurry during runs 3 and 4. Burkard air samplers were sited 50m upwind and at five distances downwind from the spreading source up to 200m, with 2-5 samplers positioned at each distance (depending on distance from source). The samplers were positioned at a height of 1.5m and agar plates exposed for 9 minutes on 5 and 6 September 2002.
Experiment 3. Fresh cattle slurry was spread as described above (Experiment 2) at IGER North Wyke. Burkard samplers were placed every 50m up to 450m from the spreading source, with Anderson particle samplers (which differentiate on the basis of aerodynamic particle size) also used at 200m and 400m on 18 and 19 November 2003.
Experiment 4. Separated cattle slurry was spread using a mobile irrigator on a dairy farm in Wiltshire in December 2002. The windspeed varied from 7-13 mph during the measurement periods. One sampler unit was placed upwind and 15 units downwind of the mobile irrigator, at distances of 20, 50, 150, 250 and 625m from the application source. Four experimental runs were completed, with selective media plates used to determine generic *E. coli* or *Listeria* levels, during experimental runs of 20 minutes duration.

Experiment 5. Cattle slurry was spread using a raingun on a dairy farm in Shropshire in April 2003. Sampling of the air downwind from the raingun was undertaken with samplers arranged at 600m (4 samplers), 300m (4), 150m (3), 50m (2) and 30m (2) from the spreading source, with one sampler upwind from the raingun. Similar settlement plates (selective media agar plates left open to the atmosphere, but with no air suction) were exposed at the same sampling points. Two runs were undertaken using REC selective plates and another two runs using *E. coli* selective plates. Windspeeds of 6-8 mph were measured during the sampling periods which varied from 15 – 30 minutes.

In all the experiments, aerosols were impacted on an agar plate within the Burkard air sampler (Burkard Scientific Ltd., PO Box 55, Uxbridge, Middlesex, UB8 2RT, UK) equipped with the appropriate target-selective agar medium.

5.3.3 Results and discussion

Experiment 1. REC were recovered from all the air samplers sited at 50m from the slurry spreading source and from most of the samplers at 125m (Table 1). This demonstrated that inoculating slurry with a marker strain of *E. coli* was an effective method of confirming the source of aerosol-borne micro-organisms captured downwind from a slurry spreading source. The data also indicated that to maximise the recovery of the marker strain in air samplers at distances of 125m or more from a spreading source, a sampling time of at least 9 minutes would be most appropriate.

Table 1. Recovery of REC (cfu per sampler) at different distances and sampling times

Sampling time (mins)	50m from source				125m from source			
1	1	4	4	2	ND	1	ND	ND
5	14	12	18	7	2	1	3	1
9	8	5	26	4	4	3	3	5

ND= Not detected

Experiment 2. During runs 1 and 2, concentrations of enterobacteria in the dairy dirty water were 9.3×10^4 cfu/ml and during runs 3 and 4, REC concentrations in the cattle slurry were 2.5×10^5 and 6.2×10^5 cfu/ml, respectively.

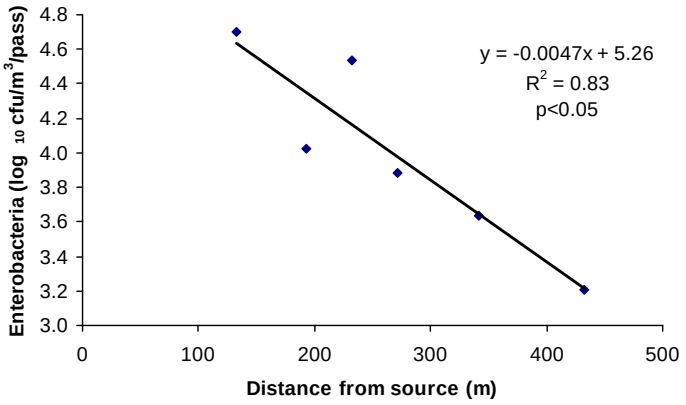
Aerosols were visible from all of the tanker runs at distances of up to 20 metres from the spreader. However, micro-organisms were detected at distances of up to 200m on each run, indicating that they were likely to be transported even greater distances (Table 2). REC added to the cattle slurry during runs 3 and 4, and detected at each distance, confirmed that the source was the spreading tanker. There was no evidence of any decrease in airborne micro-organism concentrations between 50 and 200m from the spreading source. REC were also detected 50m upwind of the spreading source, probably due to mixing that occurred in turbulent air movements created by trees (about 20m high) that were 100m upwind of the spreading source.

Table 2. Manure analysis, environmental conditions and microbial recovery during Experiment 2

	Run 1	Run 2	Run 3	Run 4
Slurry origin	Dirty water	Dirty water	Cattle slurry	Cattle slurry
pH	7.44	7.44	6.77	6.77
Dry matter (% w/w)	0.7	0.7	2.9	2.9
Tanker passes	1	2	6	6
Sampling time (mins).	9	9	9	9
Sampled air volume (litres)	180	180	180	180
Wind speed hourly average (m/s)	1.31	1.31	2.29	2.29
Beaufort scale	3-4	3-4	4-5	4-5
Organism counted	Enterobacteria	Enterobacteria	REC	REC
Distance from spreader (metres)	(cfu/litre air/pass)	(cfu/litre air/pass)	(cfu/litre air/pass)	(cfu/litre air/pass)
200	19.3	11.1	0.30	0.61
150	29.2	8.6	0.58	0.60
100	12.7	6.4	0.48	0.56
50	17.7	13.0	0.57	0.68
-50 (upwind)	13.9	12.0	0.51	0.64

Experiment 3. This experiment demonstrated that at wind speeds of 4.2 m/s, micro-organisms can be transported over 400m during the spreading of cattle slurry (Figure 17), and that airborne concentrations decreased with distance from the spreading source ($P < 0.05$; $r^2 = 83\%$).

Figure 17. Airborne concentrations of enterobacteria during cattle slurry spreading (Experiment 3).



Modelled projections from experiments 2 and 3 (based on 'high' turbulence conditions and extrapolations to the zero detection of organisms) suggested that micro-organisms could potentially be transported between 500 and 1500m at wind speeds between 2.8 and 4.2 m/s. The examination of microbial counts with distance transported from the spreading source and the size of particles transported at different wind speeds, supports the conclusionthat particles associated with microbial transfers were in the size range of 5 to 30 microns.

Experiments 4 and 5. Results from these two experiments proved inconclusive and underline the difficulties that can arise with aerosol dispersion work. In experiment 4, viable *E. coli* were only isolated on one plate at 150m from the spreading source, during one of the runs. Similarly in experiment 5, viable *E. coli* were only isolated on plates in the air samplers very close to the slurry source (30 and 50m) and on the open settlement plates at the same locations. REC were not measured on any sample plates. The failure of the REC marker runs was most likely a result of the critical timing necessary to ensure the coincident arrival of the REC labelled slurry and the spreading operation. Analysis of the applied slurry at the end of the sampling runs failed to detect any REC. This suggests that, either the venturi dosing system developed to add the REC marker to the slurry

had failed, or that the REC had died-off in the slurry. The lack of success (i.e. very low *E. coli* plate counts) in experiments 4 and 5 is more difficult to explain, as the close in samplers were visually well within the plume of aerosol particles moving from the spreading source. The problem was most probably related to the functioning of the air sampling units under turbulent conditions, as the samplers are primarily designed to work in still air conditions.

5.3.4 Summary and conclusions

These experiments have shown that aerosol borne micro-organisms generated during the landspreading of cattle slurry and dirty water (using a splash-plate vacuum tanker) can travel over 200m at wind speeds of 1.3-2.3 m/s and over 400m at wind speeds of 4.2 m/s. The transmission of REC during pig slurry spreading (using a rain gun) was also measured up to 125m from the spreading source. Modelled projections from these data indicate that micro-organisms could be transported up to 1500m from a spreading source.

6. RELATIVE RISKS OF PATHOGEN TRANSMISSION

Information was drawn together from the desk-study and experimental work, and an assessment was made of the relative risks of losses of viable pathogens to the water and air environments. For water, a ‘star’ rating system was used to represent the likely risks of loss from different sources under different conditions. Where there was little data available from the scientific literature, estimates were made based on practical knowledge and expert judgement, of the ADAS/IGER/SAC project team, of the likely risks of microbial transfer.

6.1. Transmission to water

The estimated risks of pathogen transmission to the wider environment via water are presented in Table 3. The studies carried out in the West of Scotland (Aitken *et al.*, 2001) indicated that the main pathways through which the microbial contamination of surface water systems occured were: livestock excretion while drinking or crossing watercourses; contaminated runoff from farmstead hardstandings and middens etc. running directly into watercourses; surface runoff or drainflow from fields where manures had recently been applied or where livestock were grazing. A detailed survey undertaken in the Irvine and Girvan catchments in the West of Scotland indicated that 60% of the farms had significant ‘point-source’ discharges to watercourses. Additionally, insufficient slurry storage capacity leading to land application in inappropriate circumstances (e.g. when soils were at or close to field capacity), where surface runoff or drainflow were likely to occur soon after application, was also seen to constitute a problem.

There are little available data for England and Wales on the number of farms with significant ‘point-source’ discharges from uncontained farmstead runoff. However, it was possible to obtain an indication of ‘point-source’ discharges from dairy and beef hardstandings from Defra project WA0523 (Survey of Runoff and Emissions from Hardstandings), which indicated that 80-90% of runoff from farmstead hardstandings on dairy farms and *c.*50% on beef farms was collected, with the remainder most likely seeping into proximate fields and ditches (Hatch *et al*., 2004).

As livestock typically graze in the fields for *c.*180 days/year, this activity represents a significant risk of microbial pollution where surface runoff or drainflow occur during or shortly after the cessation of grazing. Also, grazing emissions largely occur during the late spring-early autumn when they can impact directly on bathing water quality (the bathing water season in England and Wales runs from 15 May – 30 September). Similarly, the landspreading of manures (particularly fresh slurry) can present a high risk of microbial pollution where surface runoff and drainflow occur shortly after land application.
Table 3. Estimated risks of pathogen transmission to the water environment

* = low risk ***** = high risk

Operation	Risk conditions	Manure type
Land application of manures	• Direct application of manure into a watercourse ***** • Underdrained soils at or close to a field capacity, with rainfall within 7 days causing drainflow **** • Land with moderate/steep slopes at or close to field capacity, with rainfall within 7 days causing runoff / interflow***; and where adjacent to a watercourse**** • 'Dry' soils, where drainflow/surface runoff/interflow is unlikely within 1 month *	Slurry (fresh) ***** Dirty water/ slurry (stored) **** FYM (fresh) *** FYM (stored and heated)*
Grazing	• Direct deposition of faeces in a watercourse ***** • Grazing on soils with underdrainage, where rainfall causes drainflow **** • Grazing on land with moderate/steep slopes, where rainfall causes runoff/interflow***; and where adjacent to a watercourse**** • Grazing on 'dry' soils, where drainflow/surface runoff/interflow is unlikely to occur during or soon after the end of grazing*	Sheep ***** Outdoor pigs ***** Cattle ****
'Point-source'	• Uncontained farmstead hardstandings runoff ***** • Uncontained runoff from 'wet' (and fresh) farmstead middens *** • Field manure heaps ** • Runoff from tracks used by cattle ** • Slurry stores * to *****	Slurry ***** Dirty water **** FYM (fresh) *** FYM (stored and heated)*

6.2. Transmission to air

For transmission to air during the landspreading of manures, the highest risks were assessed as likely to occur:

- From the use of rainguns or splash-plate tankers to spread slurry/dirty water (compared with bandspreaders/shallow injectors).
- Where fresh (i.e. unstored) slurry or dirty water are being spread.
- When windspeeds are high, as this increases transport distances.

Solid manures are likely to present a much lower risk of airborne pathogen transmission, because aerosols are not generally created during the spreading process. Also, solid manure storage prior to landspreading will reduce the pathogen content of manures, particularly where composting has occurred in the storage heaps (i.e. temperatures of > 55°C have been reached for 3 or more days) (Nicholson *et al*., 2005).

6.3. Other vectors

There was insufficient quantitative information available to compare the risks of pathogen transmission to the wider environment via other vectors (e.g. vehicles, rodents, insects).

6.4. Summary

In the above desk-study assessment, the risks of pathogen transfer were estimated for different loss routes. However, it was *not possible to make absolute comparisons* between the different loss routes (i.e. a 5-star loss risk assessment following manure spreading does not necessarily directly equate to a 5-star loss risk assessment during livestock grazing or from point-sources). Also, individual sources of pathogens will often give rise to risks by more than one route (e.g. the landspreading of manure presents a risk of pathogen transfer via water and aerosol dispersion). Overall, it is likely that the risks of pathogen transfer to the wider environment are considerably higher via water than air, and that the risks via other vectors are relatively low.

7. PRACTICAL RECOMMENDATIONS

Pathogen control measures in place in the UK and in other countries to minimise the risks from livestock manure management to the food chain were reviewed by Nicholson *et al.* (2000). The measures identified included the manipulation of diet and dietary additions, minimum storage periods (typically 90 days) for slurry and solid manures, avoidance of recontamination of stored manure, slurry treatment, solids composting, landspreading methods, minimum harvest intervals, minimum grazing intervals and general good practice.

Chambers (2003) assessed the pathway reduction potential of these approaches by reference to existing literature and research work funded by the Food Standards Agency (Nicholson *et al.,* 2002). The approaches were combined into a series of measures grouped under diet/housing, manure storage and treatment, manure spreading and livestock grazing, and 'point sources' (Table 4). The effectiveness and reliability of the suggested measures to reduce microbial pathogen loads was assessed, both in terms of maximum reduction potential (from one to six $\log_{10}$ reduction) and reliability (i.e. is the process likely to be easily controllable under typical farm conditions). The results are summarised in columns three and four of Table 4. Measures associated with extended storage periods or the treatment of solid manures and slurries generally gave the best reductions in pathogen loads, ranging from two to six $\log_{10}$ reductions. To minimise aerosol borne microbial transfers, the use of slurry bandspreading/injection techniques (compared with raingun or splash-plate applications) will considerably reduce transmission risks to the air environment.

8. RECOMMENDATIONS FOR FURTHER WORK

The study has identified the following ***high priority*** gaps in knowledge on pathogen transfer processes and pathways to the water and air environments, which need to be addressed in future research:

8.1. Transport to water

We recommend that the main priorities for future research work should be:

- To establish the **processes and pathways of pathogen losses from farming systems** to surface waters, to provide robust data to underpin mitigation measures that may be required to ensure compliance with the EC Bathing Water Directive in certain catchments, the Shellfisheries Directive and to protect irrigation water quality.

Specifically, we need to identify the *relative contribution* of different potential pathogen loss routes to the overall pathogen burden of surface waters. Sources to be measured should include grazed pastures, fields where manures have recently been applied, farmstead hardstandings, livestock paths and walkways, woodchip corrals, solid manure heaps and streams to which livestock have access for drinking, through a combination of on-farm measurements and large scale field plot studies.

Table 4. A summary of measures suggested to reduce pathogen levels in manures and their effectiveness and reliability in reducing pathogen loadings (adapted from Chambers, 2003).

Phase	Possible measures to be considered	Log $_{10}$	*Reliability*
1: DIET/ HOUSING	**1. Dietary manipulation/ microbial manipulation of pathogen load in gut**		
	a) Cattle : fresh and conserved forage, silage additives[+]	★[+]	★[+]
	b) Poultry : vaccination /probiotics/ antibiotics	★★	★★
	c) Pigs: use of copper and antibiotics	★★	★★
2: STORAGE/ TREATMENT	**2.1 Slurry**		
	a) Store slurry for 30 days.	★★	★★
	b) Store slurry for 90 days.	★★★★	★★★
	c) Provide additional slurry store(s) to avoid recontamination of stored slurry with fresh material i.e. batch storage for 90 days.	★★★★★★	★★★★★
	d) If no storage, consider treatment:		
	(1) anaerobic digestion	★★	★★★
	(2) anaerobic digestion plus pasteurisation	★★★★★★	★★★★★★
	(3) aeration	★★★	★★
	(4) additives – LIME	★★★★	★★★
	- ACID	★★★	★★★
	(5) additives – carbohydrate substrates	★★	★
	2.2 Solids		
	a) Batch store solids for at least 90 days prior to application.	★★★★★	★★★★
	b) Compost with thorough mixing : twice within first week and > 55^{O}C for 3 days.	★★★★★★	★★★★★
	c) Avoid recontamination with fresh manure (provide additional manure pad(s) if necessary).	GOOD PRACTICE	GOOD PRACTICE
3:SPREADING OF MANURES AND GRAZING ANIMALS	a) Incorporate solid manure or slurry prior to drilling.	(★)	(★)
	b) Avoid direct application of manures or direct deposition of faeces into a watercourse	GOOD PRACTICE	GOOD PRACTICE
	c) Allow 7 days between manure application/ livestock grazing and onset of runoff or drainage	★★★	★★★
	d) Allow one month between manure application/livestock grazing and onset of runoff or drainage	★★★★★	★★★★★
4: 'POINT SOURCES'	a) Contain farmstead hardstandings runoff	GOOD PRACTICE	GOOD PRACTICE
	b) Contain runoff from 'wet' (and fresh) farmstead middens/woodchip corrals	"	"
	c) Minimise runoff from field manure heaps	"	"
	d) Minimise runoff from tracks used by cattle	"	"
	e) Maintain secure slurry storage	"	"

Pathogen reduction : one star for each order of magnitude : maximum score =★★★★★★

e.g. Reliability: ★ = very variable, ★★★★★★ = totally consistent, with intermediate scores

+ Although only one ★ assigned, this approach has some potential, but more research is needed to assess this.

(★) may increase survival within the soil, but the pathogens are less likely to contaminate water systems as they are 'protected' from loss within the soil matrix.

A small number of representative farming systems should be selected which are sited in headwaters or 'mini-catchments' where farm activity data can be collected and can be confidently reconciled with microbial loss measurements to surface waters (both upstream and downstream of the sources). Ideally, the microbiological loss measurements should complement other water quality data on nutrient (e.g. nitrate-N, ammonium-N, phosphorus) and sediment losses, as part of an integrated approach to the management of water quality. *Initially, we recommend that a Scoping Study should be undertaken to identify suitable existing Defra-funded farming systems where microbiological water quality measurements could be undertaken to complement existing nutrient loss measurements.*

- To develop **models of microbial movement** in soils, both via vertical transport mechanisms and by-pass flow. Models of solute and particulate transport in structured soils, taking account of by-pass (preferential) flow mechanisms are fundamental to quantify the risks of pathogen delivery to watercourses from agricultural land, especially losses where rainfall causes drainflow shortly after the spreading of manure or during livestock grazing. Significant progress has been made in allied diffuse pollution studies, for example, pesticide and phosphorus losses, with the development of sophisticated field-scale soil profile models. Also, a number of simple catchment scale models have been developed explicitly for modelling the mobilisation and delivery of faecal indicator organisms within a GIS framework. However, this work requires further development for application at the field and catchment scale. The measurement data collected in the above farming system studies would aid the design and parameterisation of such a modelling framework, building upon existing diffuse pollution modelling systems.

8.2. Transport to air

We recommend that the priority area for further research should be:

- Quantify the influence of manure type, spreading technique and climatic conditions (e.g. windspeed, humidity and sunlight intensity) on pathogen transmission distances and duration in relation to sensitive receptors e.g. human aerosol intake, deposition on nearby crops etc.

9. OUTPUTS

Publications

Haygarth, P. M. H., Chadwick, D. C., Hobbs, P. Butler, P. S., Oliver, D., Davies, D. and Bakewell, E. (2002). Pathogen transfer from grassland soils to surface waters. *British Society of Soil Science Conference*, Seale Hayne, Plymouth University, 9-11 September 2002.

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Presentations

Chambers, B. J., Nicholson, N., Chadwick, D. R., Aitken, M. (2003). WA0804 – Investigation of Routes by which Pathogens Associated with Livestock Slurries and Manure may be Transferred from the Farm to the Wider Environment. *Review of Defra's R and D on Farm Wastes*, 10 March 2003, Great Minster House, Westminster.

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APPENDIX I

ADDITIONAL TABLES AND FIGURES

Table A1. Distances travelled by micro-organisms during manure spreading

Manure type	Application method (detection method)	Wind speed	Furthest distance	Source

		(m/s)	identified (m)	
Chlorinated wastewater	Sprinkler (Anderson samplers -glass impingers)	1.3-6.7	200	Bausum *et al.* (1978)
Raw sewage	Crop sprinkler -	Unknown	230	Schultz (1943)
Domestic wastewater	Irrigation system (Anderson samplers- glass impingers)	2-0	350	Katzenelson & Teltsch (1976)
Treated sewage	Sprinklers (Agar plates)	2.6-3.3	650	Shtarkas & Krasil'shehikov, (1970)
Wastewater	Sprinkler	Unknown	350	Sorber *et al* (1976)
Liquid manure	Sprinkler- Anderson samplers (Agar plates static impaction)	1.3 to 6.7	228 - 274	Evenden (1972)
Liquid manure	Irrigation system (Agar plates)	7-10	400	Tamasi (1983)
Cattle FYM	Flail spreader (Anderson sampler - agar plate)	1.8	0	Boutin (1988)
Cattle slurry	High splash plate (Anderson sampler- agar plate)	2.2	80 - 120	Boutin (1988)
Cattle slurry	Sprinkler system (Glass impinger)	2.2-4.4	113	Goodrich *et al*. (1975)
Pig slurry	Raingun (Anderson sampler- agar plate)	1-2.2	200 - 350	Boutin (1988)
Pig slurry	Sprinkler (Anderson sampler- agar plate)	1-2.2	90 - 130	Boutin (1988)
Slurry	Sprinkler system (Agar plates)	Unknown	154	Tamasi & Winkler (1977)
Slurry	High splash plate Low splash plate Sprinkler irrigator Raingun (Anderson samplers)	1-1.5 1-1.5 5.5-6 1-1.5	10 75 75 80	Zeller (1982)

Table A2. Manure application rates, dry matter (DM) and N analysis in drainflow experiments at ADAS Boxworth and ADAS Rosemaund (Section 5.2.2.2)

Manure	Application rate (t or m^3/ha)	DM (%)	N content (kg/t or m^3 fresh weight)	N applied (kg/ha)
Boxworth Year 1 (28/1/03)				
Pig slurry	48	1.2	3.3	159
Cattle FYM	40	33.4	5.6	225
Broiler litter	8	74.9	38.0	304
Boxworth Year 2 (5/2/04)				
Pig slurry	52.8	1.04	2.39	126
Cattle FYM	40	24.8	4.43	177
Broiler litter	15	51.5	19.1	286
Rosemaund Year 1 (27/2/03)				
Cattle slurry	40	12.7	6.3	250
Cattle FYM	40	18	5.5	220
Rosemaund Year 2, (26/11/03)				
Cattle slurry	40	5.2	3.1	124
Cattle FYM	40	21.5	6.9	276

Table A3 : Microbiological analysis of manures (cfu/g fresh weight) used in drainflow experiments at ADAS Boxworth and ADAS Rosemaund (Section 5.2.2.2)

Manure	Total coliforms	*E.coli*	*E.coli* O157	REC	Listeria	Salmonella	Campy-lobacter
Boxworth Year 1							
Prior to spreading:							
Pig slurry	680	-	-	-	23000	ND	ND
Cattle FYM	95000	-	-	-	20000	ND	ND
Broiler litter	170	-	-	-	ND	ND	ND
At spreading:							
Pig slurry	130000	-	-	215000	ND	-	-
Cattle FYM	905000	-	-	-	ND	-	-
Broiler litter	125	-	-	-	ND	-	-
Boxworth Year 2							
Prior to spreading:							
Pig slurry	1100	7000	ND	-	ND	ND	ND
Cattle FYM	470000	9000	ND	-	ND	ND	ND
Poultry manure	100	3800	ND	-	ND	ND	ND
At spreading:							
Pig slurry	81500	81500	-	78500	-	-	-
Cattle FYM	7950	2735	-	-	-	-	-
Poultry manure	1700	350	-	-	--	-	-
Rosemaund Year 1							
Prior to spreading:							
Cattle slurry	660000	-	-	-	40	ND	ND
Cattle FYM	2400000	-	-	-	200	ND	ND
At spreading:							
Cattle slurry	3600000	-	-	63500	>1000	-	-
Cattle FYM	1000000	-	-	-	>1000	-	-
Rosemaund Year 2							
Prior to spreading:							
Cattle slurry	96000	38000	ND	-	ND	ND	ND
Cattle FYM	4200000	1500000	ND	-	ND	ND	ND
At spreading:							
Cattle slurry	-	-	-	-	-	-	-
Cattle FYM	-	-	-	-	-	-	-

ND= Not detected

Table A4 : Microbiological analysis of soils (cfu/g fresh weight) used in drainflow experiments at ADAS Boxworth and ADAS Rosemaund (Section 5.2.2.2)

Manure	Total coliforms	*E.coli*	REC	*Listeria*
Boxworth - Year 1:				
Control	15	-	ND	ND
Pig slurry	8	-	ND	ND
Cattle FYM	1150	-	-	ND
Poultry manure	1003	-	-	ND
Boxworth - Year 2:				
Control	8873	5	ND	-
Pig slurry	16200	3500	2220	-
Cattle FYM	50870	370	-	-
Poultry manure	6285	243	-	-
Rosemaund - Year 1:				
Control – event 1	2837	ND	ND	ND
Control – event 2	18240	ND	ND	877
Control – event 3	900	ND	ND	ND
Cattle slurry- event 1	6822	569	18	ND
Cattle slurry- event 2	2813	27	ND	940
Cattle slurry- event 3	3133	ND	ND	ND
Cattle FYM- event 1	2333	ND	-	20
Cattle FYM- event 2	267	ND	-	223
Cattle FYM- event 3	6353	12	-	125
Rosemaund - Year 2:				
Control – event 1	23733	ND	-	-
Control – event 2	-	-	-	-
Control – event 3	5657	ND	-	-
Cattle slurry- event 1	390533	200	-	-
Cattle slurry- event 2	-	-	-	-
Cattle slurry- event 3	7535	1	-	-
Cattle FYM- event 1	4007	47	-	-
Cattle FYM- event 2	-	-	-	-
Cattle FYM- event 3	9001	1	-	-

ND= Not detected

Figure A1. *E.coli* in soil during grazing.

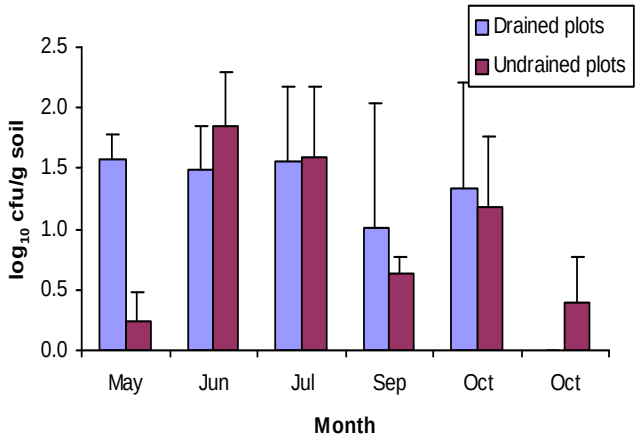


Figure A2. *E.coli* O157 in soil during grazing

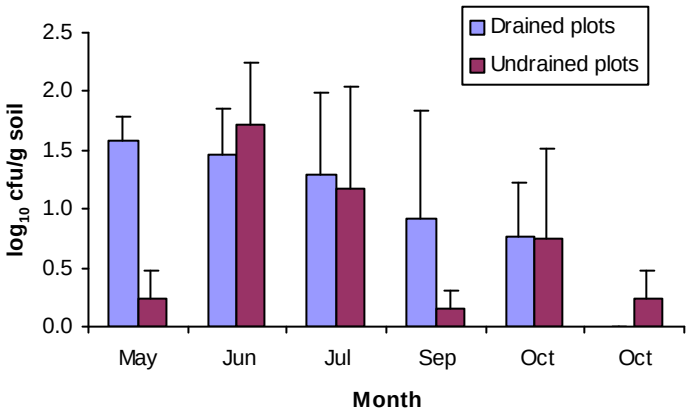


Figure A3. Suspended solids

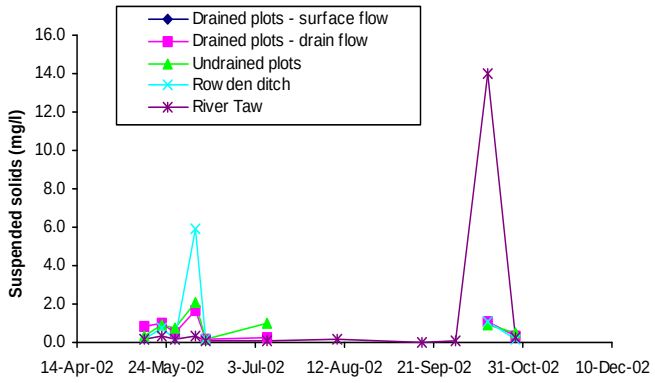


Figure A4. Turbidity

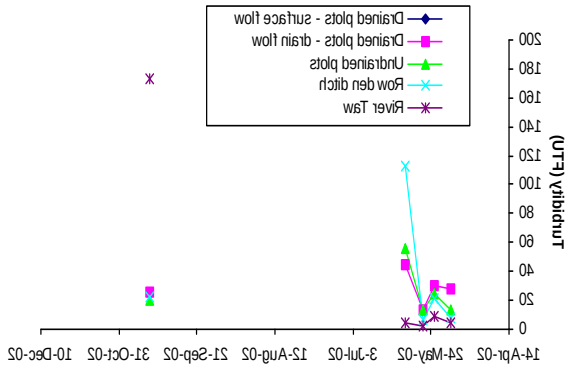
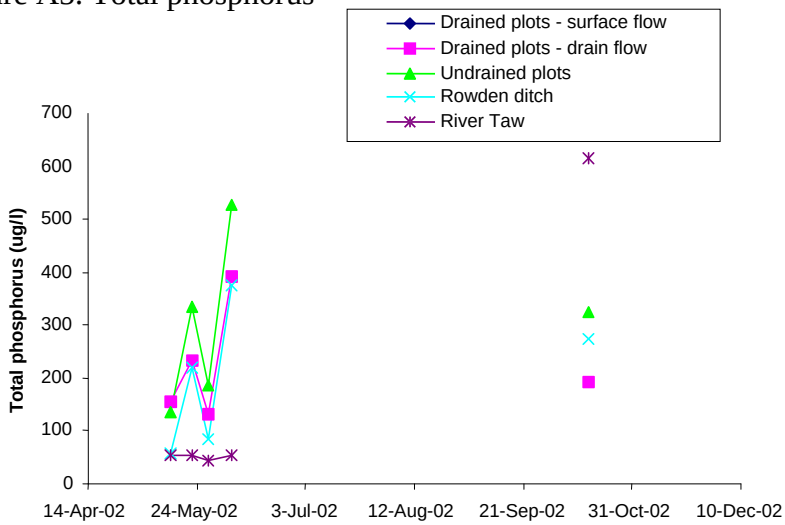


Figure A5. Total phosphorus



APPENDIX II

MICROBIOLOGICAL METHODS USED BY DIRECT LABORATORIES

Preparation of inocula

The marker organisms (REC) Escherichia coli K12, resistant to 40 µg ml^{-1} nalidixic acid, or Escherichia coli W18, resistant to 100 µg ml^{-1} kanamycin, were stored on Protect beads at –70ºC until use, and resuscitated by overnight growth at 37ºC in brain heart infusion. BHI cultures of K12 or W18, were streaked onto VRB agar containing 40 µg ml^{-1} nalidixic acid or 100 µg ml^{-1} kanamycin, respectively. Plates were incubated overnight at 37ºC. Typical colonies of K12 or W18 were inoculated into 10 ml of LB containing 1% NH_4Cl and 40 µg ml^{-1} nalidixic acid or 100 µg ml^{-1} kanamycin, respectively, to produce LB cultures, which were stored at 4ºC for up to 24 h before use. Broth cultures were prepared by initially combining 5 ml LB culture with up to 1 litre volumes of LB containing 1% NH_4Cl and 40 µg ml^{-1} nalidixic acid or 100 µg ml^{-1} kanamycin, as appropriate. Broth cultures were incubated stationary, overnight at 37ºC. Inocula were used at that stage, or, for experiments where several litres of inocula were required, were further scaled-up by combining cultures in a similar ratio.

Detection of marker organisms (REC) from inoculated slurries or waters

Slurry and water samples were diluted tenfold in MRD. Volumes (100 µl) of appropriate dilutions were spread plated onto VRB agar containing 40 µg ml^{-1} nalidixic acid or kanamycin, as appropriate, and plates were incubated overnight at 37ºC. Typical colonies were counted.

Detection of bacterial pathogens

Bacterial pathogens Salmonella, E. coli O157, Listeria monocytogenes and Campylobacter were enumerated using methods described previously (Hutchison *et al*., 2004a; Hutchison *et al*., 2004b).