Assessing the risk of the transfer of antimicrobial resistance genes between bacteria in stored and spread farm wastes

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Summary

During the storage and spreading of farm waste there may be opportunities for the transfer of genetic material between bacteria so that some acquire a wider range of resistances. Therefore a risk assessment model has been developed to estimate the risk of R-plasmid transfer by conjugation occurring between *Salmonella* Typhimurium and commensal *Escherichia coli* in slurry on a random dairy cattle farm in the UK. The model predicts that the risk of R-plasmid transfer in stored and spread waste is low, and that the risk to livestock grazing on land spread with cattle slurry is equally low.

Introduction

In the UK, approximately 90 million tonnes of farm wastes are recycled to land per annum[1]. Farm wastes have a fertiliser value and help maintain soil quality and fertility, hence their use on land intended for arable crops, livestock grazing and horticulture. It is possible for farm wastes to contain pathogenic bacteria and concern has been expressed about the possible spread of these to the human population. Initial concerns were related to the presence of pathogenic bacteria in waste and little attention was paid to the possible dissemination of antimicrobial resistant bacteria and transferable resistance genes such as conjugative R-plasmids. However, it may be possible that mobile genetic elements carrying resistance genes are transferred between bacteria during the storage and spreading of waste so that bacteria acquire a wider range of resistances than initially present. If these transconjugants can survive the storage and application process, and have the potential to colonise humans or other animals, then a new multiple resistant epidemic strain may be created.

Objectives

The aim of the work was to develop a quantitative risk assessment model to estimate the risk of conjugative R-plasmid transfer occurring between *Salmonella* Typhimurium (STM) and commensal *Escherichia coli* (EC) during the storage and spreading of dairy cattle slurry. In addition to this, the model was required to estimate the number of STM and EC transconjugants present on a random hectare of grazing land, which has been spread with cattle slurry, at the time at which cattle are turned out to pasture in the Spring. This output provides insight into the potential risk of cattle being infected with a transconjugant which could potentially be a new epidemic strain. Finally the model was required to investigate a number of practical control strategies so that it may be used to inform guidelines on best-practice for the storage and spreading of cattle slurry.

Materials and methods

The risk assessment models describe the farm storage and spreading practices of dairy cattle slurry and investigate the impact of such practices on the transfer of conjugative R-plasmids between STM and EC. Data to populate the model was obtained from the published literature, unpublished studies and where necessary expert opinion. Experimental work carried out at the Veterinary Laboratories Agency and the Health Protection Agency was used to estimate the rate at which R-plasmid transfer occurs in stored and spread waste.

To describe the transfer of conjugative R-plasmids within a random millilitre (ml) of stored and spread dairy cattle waste, a differential equation model was developed that describes both the survival of bacteria and rate of R-plasmid transfer (both of which are temperature dependent). In a storage facility since slurry is added daily, the age of the slurry, and therefore the bacterial population, will vary. Therefore a 'representative' 1ml aliquot from each addition of slurry to the storage facility is modelled over time. A similar approach is taken for waste that has been spread onto grazing land since slurry is spread at various times during the year and hence the bacterial population in a 1ml aliquot of spread waste will also vary. Although the results given below are for an average 1ml aliquot of slurry, designing the model in this way allows the worst-case scenario to be investigated, which will occur in the most recent addition/application of slurry since this is when the population of recipient and donor bacteria will be highest.

Since the aim of the model is to investigate the impact of farm waste management, it was assumed that only these model parameters are stochastic. The parameters relating to the management of farm waste include storage capacity, time for which waste is stored, time of turnout and the time between the last spreading of waste and the grazing of animals. These parameters allow the model to investigate the impact of control strategies relating to waste management and hence the model can be used to guide farmers on how to reduce the risk of R-plasmid transfer in their cattle slurry and hence the number of transconjugants on grazing land at the time of turnout.

Results

From the 1st October to a fixed turnout time (31st March), the mean probability of a recipient STM in stored slurry acquiring a conjugative R-plasmid was estimated to range from 0 to 1.62×10^{-7}. Similarly, the mean probability of a recipient EC acquiring a conjugative R-plasmid ranged from 0 to 2.03×10^{-8}. In a ml of slurry spread onto grazing land during the same time period as above, the mean probability of a recipient STM and a recipient EC acquiring a conjugative R-plasmid was slightly greater compared to stored waste because the mean probability of acquiring a R-plasmid ranged from 0 to 2.68×10^{-7} for STM and from 0 to 3.44×10^{-8} for EC.

The model also estimates the number of STM and EC transconjugants on a random hectare of grazing land, which was been spread with waste, at a (non-fixed) time of turnout for cattle. At turnout, the probability of a random hectare being contaminated with 1 or more STM transconjugants is estimated to be 0.317 and, similarly, 0.139 for EC transconjugants. However if the grazing land is contaminated, the mean number of STM transconjugants and EC transconjugants present on a hectare of grazing land is 371 (1, 1320) and 11471 (1, 22033) respectively .

Using the model, practical control strategies can be investigated, one of which is to increase the allowable length of time between the last spreading of waste onto grazing land and the turning out of cattle (defined as *T**). Table 1 shows that the probability of a random hectare (defined as *P*), that has been spread with slurry, being contaminated with 1 or more transconjugants decreases as *T** increases. Likewise if the hectare is contaminated, the number of STM or commensal EC transconjugants present decreases as T* increases.

Table 1: Probability of a random hectare, which has been spread with cattle slurry, being contaminated with >1 transconjugants and, if contaminated, the number of transconjugants when the time between last spread and turnout is at least T*.

Time T* (weeks)	***S*. Typhimurium**				**Commensal *E. coli***			
	P	**5th %ile**	**Mean**	**95th %ile**	**P**	**5th %ile**	**Mean**	**95th %ile**
1	0.682	4	3107	12096	0.571	4	70603	294466
2	0.603	2	612	2227	0.443	3	1719	6678
3	0.507	2	156	644	0.303	2	70	256
4	0.360	1	33	125	0.101	1	4	10
8	0	N/A	N/A	N/A	0	N/A	N/A	N/A

Discussion

From the above results it can be seen that the mean probability of a STM or commensal EC acquiring a conjugative R-plasmid is low in both stored and spread slurry. This is due to the decreasing number of bacteria within a 1ml aliquot of stored and spread waste over time and also that the chosen time period is during winter when the temperature is colder (the rate of R-plasmid transfer is temperature dependent).
In infectivity experiments, it is suggested that a dose of more than 10^8 *Salmonella* is required to infect calves, with a higher dose necessary to infect adult cows[2]. Although, under farm conditions, outbreaks of *Salmonella* can occur with a lower exposure the dose required is likely to be significantly higher than the mean number of STM transconjugants estimated to be present on a contaminated hectare of grazing land. Given the above, and typical management practices, it is therefore concluded that the risk of grazing cattle becoming infected with a STM transconjugant is low, however this risk can be reduced further by increasing the time between the last spreading of waste and the time of turnout.

References

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