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Pathogens and antibiotic residues in animal manures and hygienic and ecological risks related to subsequent land application

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ABSTRACT

The practice of spreading of livestock wastes onto land used for the production of food or animal feeds is widely regarded as the least environmentally damaging disposal method, however, the practice is still fraught with pitfalls such as N pollution of air and water and significant microbiological risks. Therefore this paper focuses on some of the latest developments that provide new insights into the microbiological safety of animal manures, the related treatment options and the spreading the products onto land. In conclusion the paper stresses the need to fully address issues concerning environmental contamination and transmission of antimicrobial-resistant bacteria through livestock manure, improve current environmental regulations regarding manure management practice and coordination of research activities and dissemination of technical information.

1. Introduction

With the increasing human population livestock farming has become more intensive. This has been associated with raising animal stocking densities and increased risk of transmission of diseases. It also necessitated changes in management of livestock wastes. Introduced were new ways for the handling and treatment of animal manures developed to enable increasing productivity in animal husbandry and associated especially with increasing use of litterless systems of animal rearing and the related shift from solid manure to slurry-based manure. Such changes have raised concern about an increased survival of zoonotic agents in animal wastes and their possible transmission to other animals or their contamination of the human food chain.

In relation to this, and concerning the aspects of altered epidemiology for important infection diseases, one should not forget the issue of global warming which may result in many rather abrupt and unpredictable changes in disease vectors impacting on new areas where they did not exist before. Climate change may also evoke drastic changes in traditional systems of animal rearing (e.g. keeping animals inside instead of outside because of frequent heavy rainfalls).

In relation to intensive livestock units, there is also concern about presence of antibiotics or some other pharmaceutical residues in animal manure that may pass to environment, particularly to surface water after application of these materials onto land. Moreover, the use of antimicrobial agents is related to the risk of producing antimicrobial-resistant bacteria and their subsequent release to the environment from stored or applied animal wastes.

In addition to hygienic aspects, the intensive systems impact on the environment particularly as sources of emissions of gasses and by the leaching of some nutrients to soil and water. The EU IPPC Directive (integrated pollution prevention control) takes care of the biggest units but there are also those of smaller size but with sufficient capacity to produce local or even regional problems.

Combined, all hygienic and ecological risks connected with utilization of animal manures are complex and would not be sufficiently explained to cover all the aspects in one short review. Therefore, we will focus on some latest development and information that provide new insight into the safety of treatment of animal manures and spreading the products onto land and to some challenges related to these developments.

2. Animal manure as a medium of spreading of significant pathogens

The application of livestock faeces and wastewater in agriculture may result in a public health threat only if all of the following prerequisites concur:

(a) if an infective dose of an excreted pathogen reaches the pond or a natural water body, or if the pathogen multiplies in an

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intermediate host residing in the pond or in the aquatic environment to form an infective dose;
(b) if this infective dose reaches a human host through contact or consumption of the aquacultural products;
(c) if this host subsequently becomes infected.

However, one should consider also the fact that some bacterial pathogens may multiply in the environment, so even less than an infective dose may be a threat.

A wide variety of pathogenic viruses, bacteria and parasites may be found in the faeces of wild and domestic animals and humans. When they reach water and soil, many of them have the potential to infect humans and domestic animals, sometimes when present only at low levels. These zoonotic pathogens are of great concern to the public, who are usually exposed through consumption of fecal contaminated food or water (Olson, 2003). They may also be of great concern for animals, both domestic and wildlife.

Properly handled and treated manure is an effective and safe fertilizer but untreated or improperly treated manure may become a source of pathogens that may contaminate soil, fresh products, surface and ground water and drinking water supplies (Vanotti et al., 2007). Solid livestock manure is perceived as relatively safe due to increased temperatures that can develop in this material during its storage which contribute to some reduction of pathogen numbers. This process is known as composting or aerobic digestion and it will occur only under certain conditions of the material. Many experiments were conducted on the survival of pathogens in animal wastes. However, when interpreting their results we should be careful because there are many factors that could change completely the picture. We will point to only some of them.

Firstly, we should be aware of great variability of animal manures even within individual animal species and of constant changes in their composition affected by many factors. For example, it is not clear how fresh faeces, laboratory-prepared wastes or solid wastes relate to liquid livestock wastes, which can contain unspecified amounts of faeces, urine, bedding, water, feedstuffs, blood, detergents, antibiotics and nose, throat, mammary gland and vaginal secretions (Mawdsley et al., 1996; Pell, 1997).

Secondly, the survival of pathogens in animal manures and manure slurries is often studied under controlled laboratory conditions. Kudva et al. (1998) noted that survival of pathogens in laboratory studies were generally lower than those observed in field studies. This indicates that laboratory experiments may provide a under-estimate of pathogen survival in on-farm conditions. Future efforts should concentrate on measuring the survival of pathogens in the environment.

Thirdly, some studies follow the survival and recovery of pathogens that occurred naturally in the manure while others inoculated by using various strains (or by using recently recovered livestock pathogens) and investigated their survival. Moreover, the methods of recovery and detection can vary considerably. How much difference in procedure can we accept whilst still considering the results still comparable?

Current methods for detecting pathogens in environmental systems may limit the ability to determine survival times in difficult media. Specific soil or manure properties may limit detection of pathogens when using cultivation techniques. For instance, upon being stressed, bacteria may die or adapt using a number of mechanisms including formation of spores, or entering viable but not cultivable states. In storage tanks, bacteria may become aggregated after attaching to each other or to small particles in the waste and produce single colonies, even after the death of some individual cells in the aggregate. Different waste types, and indeed different batches of the same waste type are likely to cause bacterial aggregation to differing degrees. Natural variability of the pure culture inocula and/or of bacteria persisting in the wastes over time could contribute to some individual cells adapting to the waste environment over the course of the experiment, and surviving for longer than other cells (Hutchison et al., 2005).

3. Survival of bacterial pathogens in manure

The bacterial pathogens, as may show up in manure, that are the most important with regard to human health include, *Salmonella* sp., *Escherichia coli O157 H7*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Clostridium perfringens*. *Salmonella* are *Enterobacteriaceae* which are widely distributed in the environment and include more than 2000 serotypes (Venglovsky et al., 2006). It is a well established fact that bacterial pathogens may persist for long periods in animal manures under typical farm conditions. This may be extended when the temperatures are low, moisture remains optimal, and aeration is not used. For instance, *Salmonella* and *E. coli* O157:H7 survived for 4–6 months in animal manures and slurries kept at 1–9 °C, which is up to 49 times longer than at 40–60 °C. Nicholson et al. (2005) determined that aeration of the solid manures decreased survival times for *E. coli* O157:H7 and *Salmonella* by as much as 88%. Maintenance of high dry matter content decreased the survival time. Kudva et al. (1998) noted similar changes in *E. coli* 0157:H7 in sheep manure, which survived for 630 days at temperatures below 23 °C when not aerated but only 120 days when aerated, the difference likely due to drying of the aerated manure. Paluszak and Olszewska (2002) observed survival of *Campylobacter coli* in slurries for long periods of up to 20 weeks. According to Nicholson et al. (2005), *Campylobacter* survived in stored slurries and dirty water for up to three months. Overall, *Campylobacter* numbers in the wastes fall significantly faster ($P < 0.05$; *D*-value 10.7 days) than those of the other bacterial pathogens (Hutchison et al., 2005).

McGee et al. (2001) investigated survival of *E. coli* 0157:H7 in bovine slurry from cattle fed two different diets (silage and silage plus concentrate). Examination of samples inoculated at a level of $\log_{10}6.0$ CFU g^{-1} showed 3.5 and 5.5 log reduction, respectively, in slurry over a 12 week storage period in the laboratory at 10 °C. The persistence of *E. coli* 0157:H7 in slurry over a 3 month storage period indicated the potential of the organism back to the environment although it may not represent a major source of transmission.

Previously, it was reported that *Salmonella Dublin* or *S. typhimurium* P6 survived longer in cattle slurry diluted to 5% dry matter (DM) than in very diluted (1% DM) slurry (Jones, 1980). The phenomenon of drying itself (rather than DM content) is probably more influential on bacterial behaviour in solid livestock wastes (farmyard manures) (Himathongkham et al., 1999).

The destruction of the bacteria is accelerated by increasing temperatures. In the solid fraction of pig slurry, *S. typhimurium* survived for 26 days in summer but 85 days in winter (Plachá et al., 2001), and coliforms were reduced by 90% in 35 and 233 days during the summer and winter time, respectively.

4. Survival of pathogens in manure

Parasitic protozoan survival in animal manures may also be related to temperature, but the trends are not as pronounced as those reported for bacterial pathogens. This is likely due to their ability to form cysts and oocysts for protection from environmental pressures. These parasites have been shown to be susceptible to temperature extremes, with reported survival of *Cryptosporidium* oocysts ranging from 1 h at −70 °C, 1 day at −20 °C, one or more years at 4 °C, 3–4 months at 25 °C, 1–2 weeks at 35 °C, and just minutes at 64 °C (Fayer and Nerad, 1996). *Cryptosporidium* oocysts in manures may also be susceptible to desiccation and bacterial attack whereby warmer temperatures may accelerate the degradation process. A similar pattern exists for *Giardia* cysts, but they are inactivated more rapidly than *Cryptosporidium* oocysts and are less resistant to temperature extremes.

Olson (2003) noted that the eggs of *Ascaris suum,* a common parasite in swine, are highly resistant to inactivation in feces, potentially remaining infectious for years. This is very important also with regard to the fact that *A. suum* is a zoonotic parasite. However, these environments may also be hostile, as they may harbor both predators and competitors, or produce toxic components that may reduce the pathogen viability. For instance, free ammonia, naturally produced by hydrolysis of urea and in decomposing manure, can be biocidal at high concentrations, and has been exhibited to be directly proportional to *Cryptosporidium* oocyst inactivation (Jenkins et al., 1998, 1999). Animal manures and manure slurries may remain significant reservoirs for subsequent environmental contamination by zoonotic pathogens for many months.

Raising the pH is one option for sludge treatment, but the reported effectiveness of this option varies greatly. Factors that may contribute to this variability include temperature, the type and dose of alkalinizing agent, the maximum pH attained, and the pH profile during storage (Pecson et al., 2006; Varadyova et al., 2001). Polprasert and Valencia (1981) found 27% inactivation of *Ascaris* eggs at 25 °C after 48 h treatment of excreta whereas in the same period Pecson et al. (2006) found 29% inactivation at 30 °C. The use of CaO by Polprasert and Valencia (1981) may have caused a temperature spike. Plachy et al. (1996) found only 4% inactivation of *Ascaris* eggs after 7 day of treatment at 21–25 °C, whereas Pecson et al. (2006) found 7% after the same exposure time at 20 °C.

5. Survival of pathogens after land application

Spreading of livestock wastes onto land used for the production of food or animal feeds is widely regarded as the least environmentally damaging disposal method. However, the practice is still fraught with pitfalls. It can lead to emission problems as nitrogen in the form of volatile ammonium is lost to the atmosphere, or nitrogen converted to nitrate and a pollutant of watercourses (Burton and Turner, 2003; Chantigny et al., 2004; Bernal et al., 1993). Traditionally, because the environmental aspects of nutrient surpluses, prevention of such pollution is the most important consideration during waste disposal. However, there are also significant microbiological risks which need to be taken into account when animal wastes are spread onto land subsequently used for crop production or livestock grazing (Burton and Turner, 2003; Hutchinson et al., 2005). Precautions aimed at preventing the spread of livestock-associated pathogens on farm may be helpful in limiting the spread of livestock-associated pathogens further along the food chain (Nicholson et al., 2007).

When manures are applied to land, there will be some movement of the pathogens through the soil matrix, both vertically and horizontally. The degree of mobility will affect the likelihood of pathogens reaching aquifers or surface waters. If these waters are subsequently used for irrigation of produce or for consumption by livestock, there are implications for food safety. Factors known to influence the horizontal movement of pathogens across soils include soil type, soil water content, amount and intensity of rainfall, temperature, nematodal activity, surface charge and size of microorganism, transport through plant roots, and soil pH (Cools et al., 2001; Mawdsley et al., 1995; Vanotti et al., 2005). Factors influencing the vertical movement of pathogens through the soil include the amount and intensity of rainfall, the proximity of the pollutant source, agricultural practice, weather, and the season of application (Mawdsley et al., 1995; Papajova et al., 2002). Generally, pathogen survival is favored in aqueous environments, and thus water availability and movement are the most important factors and are affected by moisture retaining properties of soils. Temperature is also an important consideration, with higher temperatures, reducing pathogen survival (The Humanure Handbook, 2005).

Within 24 h of spreading the fresh wastes onto pasture, ammonia volatilization can lower the crop-available nitrogen by >98% (Albihn and Vinneras, 2007). This result is particularly noteworthy because it shows that fresh livestock wastes are a poor source of nitrogen for grassland if the material is broadcast spread (rather than injected) onto the surface of a pasture.

Both the survival and growth potential of pathogens vary considerably between different species and subtypes of microorganisms (Mitscherlich and Marth, 1984).

According to Gessel et al. (2004) controlling manure application rate may be a means to reduce the risk of some pathogens moving with runoff.

Parasites, spore-forming bacteria, and some types of viruses generally persist for the longest periods of time in the environment. Oocysts were hardier than the bacteria. Natural inactivation factors also vary considerably due to climate, season, vegetation, soil type, method of application to land, etc. (Nicholson et al., 2005).

In general, it has been reported that survival of pathogens in soil increases when manures are incorporated into soils rather than left on the surface which may be related to decreased exposure to UV radiation, temperature extremes, and desiccation and increased availability of nutrients (Hutchison et al., 2005). However, soils may harbor competitor organisms and predators that can also reduce pathogen survival. Survival of pathogenic bacteria within soils may also be limited by low soil pH (Jamieson et al., 2002). Examination of the effects of freeze–thaw events on the inactivation of *Cryptosporidium parvum* oocysts in soil showed that oocysts subjected to freeze–thaw cycles had inactivation rates not significantly different from those subjected to −10°C under static conditions. The results indicated that 99% of oocysts exposed to soils that are frozen at −10°C will become inactivated within 50 days whether freeze–thaw cycles occur (Kato et al., 2002).

Even when not incorporated into soils, the survival of pathogens following application of manures to land may be long. Manure enhanced the attachment of *C. parvum* oocysts to soil particles. The maximum attachment was observed with 0.1% manure and it was partially reversible (Kuczynska et al., 2005).

Although the transmission process and the risk is low, there is a definite potential for *Giardia* and *Cryptosporidium* contamination of ground and surface waters from livestock operations. There are major concerns with applying fresh animal manure to fertilize agricultural land because of the potential for faecal pathogens to reach surface and/or groundwater (Olson et al., 2004).

Most zoonotic agents declined below detectible levels by 64 days, except for *Listeria monocytogenes*, which is a hardy zoonotic agent and persisted for up to 128 days in some plots. Where food crops are grown in manured soils or using contaminated irrigation waters, pathogens can contaminate the surfaces of the products growth. For instance, Islam et al. (2004) identified *E. coli* contamination on carrots, lettuce, and radishes up to 120 days following application of non-composted bovine manure as a fertilizer.

Concerns about the potential risk from pathogens associated with the land application of animal wastes will continue into the foreseeable future. Over the last decade, at least one new pathogen per year that could be transmitted through the environment has been recognized as a new public health threat (WHO, 2003). This is due to many changes in food production, advances in molecular biology, and other factors. Further information is needed on concentration and survival of pathogens present in wastes and their potential re-growth after treatment or, under favorable circumstances, after spreading. Only sufficient information will allow us to use the best management practices that will ensure safety of human food chain and prevent spreading of non-zoonotic animal diseases.

6. Antibiotics in animal manure and related hygienic and ecological risks

Antibiotics are used in food animal production in two basic manners: therapeutically (typically higher doses and administered individually or as group-treatment) to treat specific diseases and subtherapeutically (typically lower doses) for increased feed efficiency or disease prevention. However, some antibiotics are not absorbed very well by the animal and quantities are passed into the urine and manure. Some see this as a public health concern, believing such drugs could then enter ground or surface water or be taken up by plants and contribute to antibiotic resistance development and/or produce adverse reactions in those with antibiotic allergies.

The actual antibiotics amount varies from compound to compound, but in some cases the amount unabsorbed is much greater than the amount absorbed.

In most livestock operations, manure is collected in pits. In some operations, manure is then transferred to lagoons for further remediation. Several studies have detected antibiotics in these environments (Aga et al., 2003; Kumar et al., 2004; De Liguoro et al., 2003).

Regarding degradation, most antibiotics become much less stable once applied to soil, but this too varies from antibiotic to antibiotic. Antibiotics that bind strongly to soil and have shorter half-lives can be completely degraded within the soil and are not usually detected in ground water, surface water, or plants. For those antibiotics that bind strongly to soil yet have long half-lives, there is also a concern that these drugs could be taken up by plants.

When antibiotic is used as a feed additive, the antibiotic dose varies from 3 to 220 g per tonne of feed depending on the type and size of the animal and the type of antibiotic (McEwen and Fedorka-Cray, 2002). It is claimed that even the recommend low doses of antibiotics given as growth-promoting and prophylactic agents still encourage the development of antibiotic-resistant bacteria (Khachatourians, 1998).

Hirsch et al. (1999) analysed various water samples from agricultural areas in Germany for 18 antibiotic substances and observed no contamination except for two sites. They concluded that intake from veterinary application to the aquatic environment is of minor importance.

In 1997, the USDA estimated a livestock population of more than eight billion animals (more than 95% being chickens and turkeys) producing up to 132 billion tonnes of manure in the United States. Considering that the antibiotic dose varies from 3 to 220 g per Mg^{-1} of feed and a substantial amount (even 90% of some antibiotics) of them ends up in manure the presence and persistence of antibiotics in this large quantity of manure presents a significant environmental problem, both in terms of direct toxicity of these antibiotics to soil microflora and fauna as well as potentially increasing antibiotic resistance in the environment. Baguer et al. (2000) claim that land application of antibiotic-laced manure appears to be the main pathway for the release of antibiotics in the terrestrial environment.

Until recently, research on antibiotic use has been mainly directed toward their beneficial and adverse effects on the intended end user, humans and animals. However, there have been relatively few studies on the effect of these antibiotics in the wider environment including possible uptake by plants from manure-amended soils. Consumers may unknowingly be ingesting traces of some of these antibiotics when they eat vegetables grown on manure-applied lands.

In this study by Christian et al. (2003) all liquid manure samples from two investigated farms, cattle farm and cattle and swine farm but with separate storage tanks for the different liquid manure, contained quantities of antibiotics in measurable concentrations of a few milligrams per kilogram. In one case a sample of fresh swine manure taken from a store located under the building contained 20 mg kg^{-1} sulfadimidine. In soil fertilized with swine liquid manure and in soil fertilized with cattle liquid, sulfadimidine could also be detected in the upper 20 cm layer, three months after land application. Tetracyclines could not be found in manures and soils in quantifiable concentrations. Only in one pure cattle manure there seemed to be chlortetracycline of estimated 0.1 mg kg^{-1}, and in one swine liquid manure concentrations less than 0.01 mg kg^{-1} can be held for ciprofloxacine and for enrofloxacine. The authors did not present information on administration of antibiotics on the investigated farms. The investigated soil samples showed none of the investigated antibiotics, which was not astonishing, because tetracyclines are known to bind strongly to soil particles (Christian et al., 2003).

Watabe et al. (2003) examined the prevalence and diversity of bacterial faecal pathogens in slurry, slurry solids and liquid fractions from a commercial pig farm in Northern Ireland and observed that *Salmonella* species were identified in all slurry samples. The majority of *Salmonella* isolates (57.7%) displayed antibiotic resistance to at least two antibiotic agents, 34.6% of isolates to three agents and the remainder (7.7%) was resistant to four antibiotics. Resistance was developed to tetracycline (100%), sulphonamides (84.6%), furazolidone (38.5%), nalidixic acid (15.4%) and streptomycin (15.4%).

Results obtained in the study by Duriez and Topp (2007) that there are factors beyond short-term on-farm antibiotic use that influence the frequency of antibiotic resistance and patterns of multiple antibiotic resistance in bacteria shed by livestock. The role of environmental contamination from livestock wastes in promoting antibiotic resistance is difficult to evaluate against the background of the high frequency of resistance to antibiotics found in soil bacteria (ĎCosta et al., 2006; Sengelov et al., 2003). Nevertheless, prudent use of antibiotics, particularly with respect to the chronic provision of growth-promoting agents and the use of antibiotics that are important for human and animal health is advised (McEwen, 2006).

Determining the fate of unabsorbed antibiotics excreted in livestock manure is a relatively new field of study. With only a small number of studies, it is difficult to determine whether antibiotics found in livestock manure have an effect on human health once they are released to the terrestrial environment. A compilation of data from several studies, however, suggests that with each manure remediation step (e.g., digestion in lagoons, degradation in soil, etc.) the amount of detectable antibiotic drops significantly. As such, the concentrations of antibiotics found in sources that could impact human health such as ground or surface water, or plants, are at least an order of a magnitude lower than the concentrations judged by the Center for Drug Evaluation and Research to represent an appreciable risk.

However, there is another aspect to the agricultural use of antibiotics, the occurrence of resistant bacteria. Although the latent period between the introduction of an antimicrobial and the emergence of resistance may vary, once the prevalence of resistance in a population reaches a certain level, reversal of the problem may be extremely difficult (Swartz, 2002). Repeated exposure of bacteria to antimicrobial agents and access of bacteria to increasingly large pools of antimicrobial resistance genes in mixed bacterial populations are the primary driving forces for emerging bacterial resistance. The use of antimicrobial agents inevitably selects for resistance of both commensal and pathogenic micro-organisms exposed to the agents. Similarities in patterns of resistance in *E. coli* were observed in livestock animals and environmental samples taken from their respective farms.

Transmissions of antibiotic resistant genes from manure bacteria to pathogen bacteria have been conducted in the laboratory. Indeed, there is a potential risk of antibiotic resistance transfer but the extent of the risk is unknown. Most antibiotic resistance in humans is from inappropriate use by physicians and patients (Olson, 2003).

The presence of zoonotic pathogens in the environments may begin with the stocking of infected animals or to the use of selected feed products on the farm. Animal feeds and drinking water containing antimicrobial compounds may lead to the development and persistence of resistant bacterial zoonoses and non-zoonotic agents in livestock animals which may proliferate through the farm environment.

7. Conclusions

Much work is still needed to fully address issues concerning the contamination of our environment and antimicrobial-resistant bacteria and transmission of zoonotic and non-zoonotic pathogens through livestock manure. We have to highlight that infection by zoonotic pathogens results not only in extensive human suffering but also in significant economic losses. Because of the continuing problems caused by contamination of food and water resources with zoonotic agents we believe that current environmental regulations and conventional animal manure management practices are inadequate for protection of human health and the environment. There is a need to improve the coordination of research activities and dissemination of technical information, methodologies and new technologies between research scientists and a vast array of end users such as education institutions, regulatory bodies and farm owners.

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