THE ANALYSIS OF REAL MICROBIOLOGICAL RISKS FOR DISSOCIATED SLURRY

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Abstract

Aim of this article has been to inform about pathogenic microorganisms in manure and slurry, factors of their survival and methods of their reduction in manure and slurry. Mainly bacteriological risks of cattle waste use and their solution are interpreted in this review.

Key words: cattle waste, disease risks, reduction of pathogenic microorganisms

INTRODUCTION

Solid wastes of cattle operations contain bedding materials such as straw, sawdust or sand that contribute to the aeration of wastes while liquid slurry with the lack of bedding is rather anaerobic (Strauch, Ballarini, 1994). A mixture of liquid and solid cattle faeces contains a wide spectrum of microbial agents and is their multiplication medium at the same time (Catanzaro, 2000).

Fresh faeces can contain a high count of bacteria, 10^9 or 10^{10} CFU/g as determined by conventional culture methods (Salanitro et al., 1977), or 10^8 CFU/g comprising only aerobic bacteria (Krueger et al., 2002). If farmyard manure is stored before its application for a long time, the count of intestinal pathogens will decrease in the meantime. The typical decimal reduction time in manure and agricultural soil tends to be several days at 20 to 40°C and several weeks at 4 to 10°C (Himathongkham et al., 1999; Placha et al., 2001; Hutchison et al., 2005a). Mainly bacteriological risks of cattle waste use and their solution are interpreted in this review.

Pathogenic microorganisms in manure and slurry and factors of their survival

Cattle faeces may contain many human and animal pathogens such as *Salmonella spp.*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Mycobacterium avium subsp. paratuberculosis* (*Mycobacterium paratuberculosis*), *Cryptosporidium parvum and Giardia spp.* (Pell, 1997). As for these pathogens, the knowledge of *Mycobacterium paratuberculosis* persistence in the system of cattle faeces treatment is poorest. It is due to the low rate of M. paratuberculosis growth and difficult cultivation (Grewal et al., 2006). The reduction and control of microorganisms can be done by microbiological, chemical or physical methods. The efficiency similarly like advantages and disadvantages of these methods are different. Some methods can provide beneficial products, i.e. they bring about new opportunities for new sources of farm income (Heinonen-Thanski et al., 2006). The survival of pathogenic microorganisms in manure is influenced by many factors including temperature, moisture, pH, physical composition of composted material, type of bedding and microbial competition (Hess et al., 2004).

Escherichia coli O157:H7 is primarily transmitted by a food-borne route, causing diseases of humans such as haemorrhagic colitis and haemolytic-uraemic syndrome while their frequency has been increasing since 1982. Recently, the disease outbreak in humans was associated with contacts with the animal environment (Pritchard et al., 2000; Heuvelink et al., 2002; Payne et al., 2003; Varma et al., 2003). The environment of domesticated ruminants may be an important reservoir of *Escherichia coli* O157:H7 and it is a continuous risk of human exposure. *E. coli* O157:H7 is known to survive in raw manure for many months, but the pathogen may be eliminated in composting conditions similar to those that are required for the elimination of coliform bacteria (Hess et al., 2004).

Davis et al. (2005) examined the distribution and survival of *E. coli* O157:H7 in the nearest environment of individually housed cattle after their experimental inoculation; samples from the farming environment of inoculated animals were frequently positive for *E. coli* O157:H7 although no *E. coli* O157:H7 was detected in faeces and the animals were culture-negative. They also proved that bovine urine was an indispensable substrate for the growth of *E. coli* O157:H7 and that the increased growth of bacteria in the presence of bovine urine was influenced by urine temperature and concentration. These authors reported for the first time that the addition of bovine urine to bedding enhanced the replication of *E. coli* O157:H7.

Viable count methods were used to study the length of survival of pathogenic microorganisms in cattle manure during storage and following application to the soil (Nicholson et al., 2005). *E. coli* O157:H7, *Salmonella* and *Campylobacter* were surviving in stored slurry and contaminated water up to three months, and *Listeria* also survived for three months. In contrast, all these pathogens survived for less than a month in heaps of solid manure where temperatures higher than 55°C were

registered. In the following application of manure to the soil *E. coli* O157:H7, *Salmonella* and *Campylobacter* generally survived in the soil for a month while *Listeria* was surviving for more than a month.

LeJeune and Kauffman (2005) stated that *E. coli* O157:H7 persisted at higher concentrations in sawdust used as bedding (similarly like when it was used as an additive at composting) compared to sand. The choice of the material for bedding can influence the prevalence of *E. coli* O157:H7 on dairy farms. On the other hand, Miller et al. (2003), who investigated the proportions of *Escherichia coli*, coliform bacteria and the total count of aerobic heterotrophs in two types of bedding (barley straw and wood chips) in feedlot cattle, reported that bedding did not have a significant influence ($P > 0.05$) on the examined groups of bacteria while the year season did. The counts of *Escherichia coli* and coliform bacteria were significantly higher from 1.72 to 2.02 $\log_{10}$ units in summer than in the remaining three seasons, which reflected a highly positive correlation of *Escherichia coli* and coliform bacteria with air temperature. Larney et al. (2003) also compared two types of bedding (cereal straw and wood chips) in relation to the composted feedlot manure of beef cattle and they stated that the type of bedding did not affect the reduction of coliform bacteria and *Escherichia coli*. Dehydration probably influenced the elimination of coliform bacteria to a lesser extent because water losses were low (< 0.07 kg kg^{-1}) in the first seven days of composting.

Although pathogen elimination by composting was documented well (Tinquia et al., 2002), the regimes of composting (time and temperature) required for the elimination of coliform bacteria, *Escherichia coli* and other pathogens are rather variable. Schleiff and Dorn (1997) reported that it was possible to culture *E. coli* from dry poultry dung after 88-day composting. The effect of higher temperatures on a shortening of the time necessary for a reduction in pathogenic microorganisms was illustrated by Himathongham et al. (1999), who measured the D reduction value 10^5 in *E. coli* after 105 days at 4°C or after 45 days at 37°C at laboratory incubation with cattle manure.

Arrus et al. (2006) studied the effect of temperature on salmonella survival in pig slurry and seasonal temperature profiles in farm slurry storage reservoirs. The survival of salmonellas was determined in stored slurry of sows and porkers and in slurry from a piglet nursery barn stored at 4, 25 or 37°C and inoculated with a cocktail of four serovars containing *Salmonella* serovar *typhimurium*, *Salmonella agona*, *Salmonella hadar* and *Salmonella oranienburg*. Slurry temperature fluctuations were monitored in an overhead tank and in a ground storage reservoir at various depths for $< = 16$ months on two farms. The count of salmonellas in slurry dropped during storage but their survival was > 300 days in manure stored at 4°C; survival was slightly better in slurry from the piglet nursery barn. Survival was significantly shorter ($p < 0.05$) at a higher temperature in all types of manure. The decimal reduction time (D value) of salmonellas in slurry stored at 37°C was from 0.9 to 1.4 days, at 25°C from 8 to 19 days and at 4°C from 22 to 60 days. Slurry temperature in a storage reservoir in the period autumn-winter-spring fluctuated from 0.5 to 8°C in the overhead tank and from 1.5 to 11°C in the ground storage reservoir. In summer slurry temperature fluctuated from 14 to 19°C in the overhead tank and from 16 to 17°C in the ground reservoir. While *Salmonella* did not grow in pig slurry, the registered temperatures of storage reservoir could the support salmonella survival over winter and lead to field contamination during spring application.

Hutchison et al. (2005a), who studied the fate of pathogens in livestock wastes applied to fields, concluded that *Listeria monocytogenes* was the most resistant zoonotic agent surviving for 128 days before its concentration dropped below the detectable level. It is possible to routinely isolate the genus *Listeria* from the soil, and for this reason it is probably well adapted to this niche.

A decline of zoonotic agents in livestock liquid wastes stored in batches on-farm was tested by Hutchison et al. (2005b). Pathogens multiplied in controlled laboratory conditions such as *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes* and *Cryptosporidium parvum* were inoculated into the volume of 35 000 litres of fresh wastes (pig slurry, bovine slurry and contaminated water). D values for the bacteria were 6 to 44 days and for *Cryptosporidium parvum* 133 to 345 days. The decline of *Campylobacter jejuni* was significantly faster than that of the other bacterial pathogens while *Escherichia coli* O157:H7 declined significantly more slowly. On average, the bacterial decline was not influenced either by the period of deposition and storage or by the content of waste dry matter, but it was faster in contaminated water than in pig slurry. The physicochemical composition of wastes in each category significantly varied. The authors recommended to store the livestock liquid wastes if they are contaminated by pathogenic microorganisms for 6 months in order to reduce the contamination level. Such wastes should be stored in batches and should not be exposed to any continuous addition of other waste.

Grewal et al. (2006) investigated the persistence of *Mycobacterium avium* subsp. *paratuberculosis* and other zoonotic pathogens (*Escherichia coli*; *Salmonella*; *Listeria*) using three usual methods of manure treatment (thermophilic composting at 55°C, manure packing at 25°C and slurry storage in a sludge tank). Manure from the loose housing of dairy cows was artificially inoculated with pathogenic microorganisms. In manure composting and packing sawdust or straw were added to provide for optimum moisture (60%) for composting for 56 days. In the simulation of slurry storage water was added to

manure and the slurry was put in triplicate into sealed four-litre Erlenmayer flasks and incubated in environmental conditions (incubation temperature below 25°C) for 175 days. The presence of pathogens was tested on day 0, 3, 7, 14, 28 and 56. After 56 days of composting 45 to 60% of carbon were converted to CO_2 at composting while no significant changes in carbon content were found out in liquid slurry. Composting at 55°C reduced *Escherichia coli*, *Salmonella* and *Listeria* within three days. In slurry and in packed manure, i.e. at a temperature of 25°C and lower, some of these microorganisms were detectable until day 28. *Mycobacterium paratuberculosis* was detected by the standard culture only on day 0 in all types of manure treatment, but it was not detectable on day 3 and 7 in all three types of treatment. On days 14, 28 and 56 *Mycobacterium paratuberculosis* was detected only in the liquid treatment. In contrast with the results of *M. paratuberculosis* determination by culture the DNA of *M. paratuberculosis* was detected during day 56 in all three treatments, and also on day 175 in the liquid treatment. As *M. paratuberculosis* was not detectable by the culture method in compost and packed manure after day 3, it is suggested that the cells were either dead or they were present below the detection limit of conventional culture methods in these treatments.

Jörgensen (1977) reported that in anaerobic conditions *M. paratuberculosis* might survive for 252 days in bovine slurry stored at 5°C and for 98 days in slurry stored at 15°C. Grewal et al. (2006) stated that a longer time was necessary to eliminate *M. paratuberculosis* from manure in liquid storage. *M. paratuberculosis* may persist for more than two months at concentrations not detectable by culture no matter whether manure is composted, packed or stored in a liquid form in anaerobic conditions. The factors such as moisture content and manure stabilisation seem to play a role in the elimination of pathogenic microorganisms at low temperatures. The types of additives used for dairy manure composting influenced the rate and extent of organic matter conversion and the initial volume of compost density but they did not affect the survival of pathogenic microorganisms.

Studies of the survival of pathogenic microorganisms in laboratory conditions show a higher survival of pathogens because the organisms does not suffer from circadian fluctuations of temperatures, sunshine UV radiation and desiccation effect of the air current (Hutchison et al. 2000).

Watabe et al. (2003) studied the prevalence of bacterial faecal pathogenic microorganisms in separated and unseparated stored pig slurry. *Salmonella* spp. was identified in all components of slurry samples (unseparated slurry; separated solid fraction; separated liquid fraction of slurry) while *Campylobacter* spp. was isolated only from unseparated slurry and liquid separated fraction. In both cases the prevalence of pathogens was higher in separated liquid fraction whereas it was the lowest in separated solid fraction. None of the samples was positive for *E. coli* O157:H7, *Shigella spp*. or *Y. enterocolitica*. The study demonstrated a marked reduction in the prevalence of *Campylobacter* and *Salmonella* in the solid fraction of separated pig slurry.

Methods of reduction of pathogenic microorganisms in manure and slurry

Biological methods of microorganism reduction

Composting in heaps

Solid farmyard manure is relatively rich in dry matter because it contains bedding material. A high content of organic matter in solid farmyard manure is degraded by heat-producing aerobic respiration in the process of composting, contributing to a sanitation process. The count of intestinal pathogenic microorganisms will decline to such a low level that the risk will be minimised. Simultaneously, some proteins are degraded by ammonification, which leads to an increase in pH and ammonia level, and this increased pH value may threaten the survival of many microorganisms, also resulting in a sanitation process.

If manure is composted in heaps, the heat does not increase in all parts of manure heap in the same way because the lack of oxygen inside the manure heap usually hampers very active aerobic degradation processes and the temperature in the most distant part may be approaching an outside temperature. Moreover, manure age is different in various parts of the heap. It is to note that all traditionally established composts cannot be considered to be free from intestinal microorganisms, i.e. vegetable products applied composted bovine manure were found to contain intestinal microorganisms comprising both indicator and pathogenic microorganisms (Holopainen et al., 2002; Mukherjee et al., 2004).

Digging over of solid manure is one of the methods that will increase oxygen content inside the heap during the continual composting process and will lead to a decrease in the count of intestinal microorganisms. But digging over is a cost-intensive work when also ammonia losses increase, which reduces the ameliorating value of manure. Heap composting for 2 to 3 months in a space under a simple shelter from rain may be satisfactory if manure is designed for own use, not for special purposes. This process may ensure quite a good reduction in the count of intestinal microorganisms in bovine manure. The count of coliform bacteria declined from 10^7 or 10^8 to 100 or 1 000/g and the count of intestinal pathogens decreased below detection limits during successful composting (Tinquia et al., 1998; Larney et al., 2003) but in less successful composting 10^6 or 10^5 coliform bacteria may be found out in about two months (Mason et al., 2004); the efficient destruction of potential intestinal pathogens would not be guaranteed this time.

Composting in a reactor

Solid manure and other agricultural wastes may be composted in a reactor under a high temperature when within a several-day retention time period the counts of enterococci and faecal coliform bacteria are reduced to less than 1 000 CFU/g (less than the detection limit) and salmonellas are destroyed in composted material. The design of containers, often drums or tunnels, is adapted for operation in continual processes or in batches. The continual process is better because it is run only at the exponential or stationary phase with a high reaction rate, and so it is possible to avoid the lengthy lag phase at the start and the extinction phase at the end. Thanks to aerobic respiration the composted material may reach temperatures as high as 70°C for 60 minutes, as laid down by the EU directive (1774/2002). Many times post-composting in heaps must follow to prevent the phytotoxicity of composted material and to obtain a stable product that will be a good fertiliser and a good source of humus for soil. Post-composting is also necessary because composted products are used in spring or in summer. Commercial composting reactors are costly and energy is necessary for stirring. In a laboratory composting reactor *E. coli* O157:H7 was also destroyed at 50 to 60°C within three days (Hess et al., 2004).

Slurry aeration

Slurry contains any faecal material and waste water from machine milking and faeces disposal. Typical dry matter content is lower than 10%. It is possible to use pumping for slurry transport to avoid heavy and unpleasant human labour. Aeration is done either in batches or as a continual process. Aeration is carried out at a low temperature or at higher temperatures in a thermo-insulated reactor because the process itself generates heat. Retention time is given by the treatment method and ranges from 20–30 days (in the batch process starting in arctic winter) to 3-5 days (in the continual process at a high temperature). Aeration needs electricity for pumping and may lead to ammonia reduction according to the type of aeration equipment. On the other hand, it is possible to achieve 90 to 99.9% reduction in the count of intestinal bacteria or viruses at a low temperature (0–30°C), and odour reduction is also quite important; slurry is highly homogeneous and is easy to apply. This is the reason why slurry aeration has other positive effects on green forage, silage sanitation and yield level (Heinonen-Tanski et al., 1998; Leinonen et al., 1998).

If the process is run in a thermo-insulated continually operating reactor, thanks to aerobic respiration it is possible to achieve heat treatment at 70°C for 60 minutes. A reduction in microorganisms may be very high and the count of intestinal microorganisms is reduced to lower values than detection limits (Heinonen-Tanski et al., 2005b). If detection limits for indicators are achieved (typically 10 or 1 CFU/g or PFU/g – plaque-forming units for viruses), the presence/absence analyses for intestinal pathogenic microorganisms should give negative results and the product should not be microbiologically unsafe any longer. The method of heating to 60°C for 30 minutes seems the most suitable for virus inactivation in pig slurry (Turner and Burton, 1997).

Anaerobic treatment of slurry

Slurry contains organic matter than can be converted to methane through methanogenesis. Because the diversity of methane-producing bacteria is rather low, a continual or at least semi-continual process is preferred and the quality of raw material should be quite similar. This complicated process generates itself a small amount of energy because energy is in the final product. Specific temperature (constant temperature between 30 and 60º C is preferred) is needed for this process. The reactor should have thermic insulation and a part of produced methane must be used for the reactor heating at least during cold periods. The formation of methane is quite sensitive to disturbing factors (such as antibiotics in slurry, etc.) and the reactor is more complicated compared to equipments for the aerobic treatment of slurry.

In some cases the process of methane formation at a low temperature highly reduced intestinal microorganisms (Côté et al., 2006). The results of experimental anaerobic digestion of pig slurry in 40-litre sequential batch reactors at 20º C for 20 days were as follows: 97.94 to 100% reduction in the original populations of coliform microorganisms; 99.67 to 100% reduction in the original populations of *E. coli*; not detectable levels of the original genera *Salmonella*, *Cryptosporidium* and *Giardia*.

The count of intestinal pathogenic microorganisms can be reduced to a larger extent and hygienic risk can be minimised more easily if methanogenesis takes place at a higher temperature (more than 50ºC) (Martens et al., 1998). Another advantage of higher temperature is a higher reaction rate, allowing a smaller size of the reactor. Nevertheless, anaerobic treatment is less efficient in the reduction of enteroviruses compared to aerobic treatment (Pesaro et al., 1995), but in operating conditions methanogenesis at higher temperatures may produce adequate hygienic products. The risk of a disease will be reduced if pasteurisation is run at 70º C for 60 minutes (as set down by the European Parliament and Council of the European Union, 2002, Regulation (EC) No. 1774/2002).

Chemical methods

Mainly lime products are used as chemical compounds although it is possible to use some oxidising compounds such as peracetic acid if their price is reasonable (Heinonen-Tanski et al., 2006).

The application of lime products is based on an increase in pH, which inactivates microbial cells and viruses. The ammonia release at high pH (above 10) also acts in an inhibitory way for many intestinal bacteria. Lime

products were used for the destruction of salmonellas or other pathogens present in manure. Calcium oxide (quick lime) or calcium hydroxide are applied as lime products because of their solubility in water. When these compounds are added to manure, the present carbon dioxide will react and water-insoluble calcium carbonate will be formed. If these products are added to solid manure, manure and lime products should be deposited in thin layers so that the mixture will be as homogeneous as possible. It is recommended to add 30 kg of lime per 1 ton of solid manure. If lime is applied to slurry, stirring with a pump is efficient and the efficient concentration is only 10 kg/ton (Heinonen-Tanski et al., 2005a). For complete inactivation of Aujeszky's disease virus in pig slurry Koch and Euler (1984) reported the dose of 30 kg of lime per cubic metre of slurry and pH value at least 11.5 to achieve its efficient inactivation.

The official decree requires using a 7-day treatment but it was found out that only two days were a sufficiently long time for the destruction of intestinal microorganisms below the detection limit (Heinonen-Tanski et al., 2005a). It is not necessary to use any special equipment for lime treatment, so it can be recommended for a temporal use, e.g. for the rapid destruction of Salmonella and other pathogenic microorganisms. Slurry treatment with calcium hydroxide reduces the concentrations of pathogenic microorganisms including viruses (Derbyshire and Brown, 1979). Turner and Burton (1997) also described the application of recommended concentrations of chemicals such as calcium hydroxide, sodium hydroxide or formalin as one of the methods of virus inactivation in pig slurry.

Peracetic acid was tested as an alternative disinfection of waste waters. Morris (1993) and Baldry et al. (1991) compared a treatment with peracetic acid with chlorine treatment when chlorine was found to be more efficient in a reduction in virus titres than peracetic acid, which needed a longer time for its virocidal activity. Bacterial inactivation was similar if these two agents were used. It is also well-known that a large amount of foam is produced when slurry is treated with peracetic acid.

Hydrogen peroxide is used for disinfection of water, waste water and slurry. Compared to the other oxidants, its advantage is that it is a non-toxic, harmless and environmentally acceptable product. Tofant et al. (2006) tested the effect of the application of hydrogen peroxide with the catalytic activity of silver and iron ions in disinfection of porcine and bovine slurry. The treatment of the liquid fraction of bovine slurry with a mixture of hydrogen peroxide and iron ions at a final concentration 1.5% improved sensory, physicochemical and microbiological parameters. A similar result was obtained after the treatment of porcine slurry with a mixture of hydrogen peroxide and silver ions at a final concentration 2%. Metal ions Fe^{2+} and Ag^{+} enhance the oxidation activity of hydrogen peroxide by the formation of hydroxyl radicals.

Samples of fresh cattle manure were inoculated with pure cultures of *S. typhimurium* DT104 and *E. coli* 0157:H7 and their survival were tested after the addition of sodium hydroxide, ammonium sulphate, sodium carbonate and/or urea. A reduction in pathogenic microorganisms in manure was achieved by the combination of high concentrations of CO_3^{-2} and NH_3,

which are parameters related to pH. The addition of urea could be an easy treatment of manure based on the combination of both antimicrobial factors (Park and Gonzales, 2003).

Physical methods

Heat treatment such as pasteurisation is required as an efficient method for the destruction of intestinal microorganisms to an acceptable level of hygienic risk. Heat treatment in a pasteurisation apparatus was found to be efficient in the control of many viruses (Turner et al., 2000). This apparatus should be transportable, and its operation is essential during an epidemic breakdown (Burton et al., 1999) for the efficient destruction of pathogenic microorganisms in accordance with the EU requirements.

Burning is used in some cases. This process is applicable to relatively dry (poultry or horse) dung. As burning will destroy all nitrogen and organic matters, it should be used as the last alternative in contrast with the other methods if they do not generate a product that could be applied as a beneficial fertilizer and to increase the humus content in soil. The other physical methods such as irradiation may be considered theoretically but they are too cost-intensive (Heinonen-Tanski et al., 2006).

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