Survival of manure-derived pathogens in soil

Survie de pathogènes liés aux déjections dans le sol.

D. Cools, G. Van der Velde, R. Merckx, K. Vlassak
KULeuven, Lab. of Soil Fertility and Soil Biology, K. Mercierlaan 92,
3001 Heverlee. Belgium.
E-mail : danielle.cools@agr.kuleuven.ac.be

J. Verhaegen.
KULeuven, Lab. of Bacteriology.
UZ St-Raphaël, Capucijnevoer 33,
3000 Leuven. Belgium.

Abstract

We studied the survival of Escherichia coli and Enterococcus spp from pig manure in soil at different temperatures (5, 15, 25°C) and variable moisture contents (40%, 80%, 100% of Water Holding Capacity) and at different manure application rates (3.5, 7, 14 l/m²). The manure applied containes 10^6 to 10^7 CFU/ml of E.coli and enterococci.

Preliminary results indicated that during the first 68 days enterococci survived better than E. coli, at all temperature and moisture levels. Moisture content did not affect the survival of either species. Both survived well at a level above the detection limit (DL = 10^3 CFU/g soil), at all moisture contents at 5°C.

Keywords : survival, pathogenic bacteria, manure, soil.

Résumé

Nous avons étudié la survie des bactéries *Escherichia Coli* et *Enterococcus spp* apportées par le lisier de porc à un sol à différentes températures (5, 15, 25°C) et à différents taux d'humidité (40%,80%, 100% capacité au champ) et pour différentes doses d'apports (3, 5, 7 et 14 l/m²).

Le lisier apporté contenait 10^6 à 10^7 UFC/ml de *E-Coli* et enterococci. Les résultats préliminaires indiquent qu'au cours des premiers 68 jours, enterococci survit mieux que *E.Coli* pour toutes les températures et niveaux d'humidité testés. Le niveau d'humidité n'influence nullement la survie de chacune de ces 2 espèces. Toutes deux se situent au dessus du niveau de détection limite (DL = 10^3 UFC/g sol) quelque soit l'humidité et à 5°C.

Mots-clés : survie, bactéries pathogènes, déjections, sol.

1. Introduction

The use of antibiotics in animal husbandry, as well as research on its implications for human health began in the mid 1940's. Scientists concluded that the use of antibiotics in animals selected for resistant bacteria, may serve as a reservoir of antibiotic-resistant pathogens. A direct link to human health remained to be determined, since transfer of bacteria from animals to humans was a rare event (Sundlof, 1997). Antibiotics are nowadays used in meat production with three purposes : to cure an illness (therapy), to prevent possible pathology (profylaxis) and to promote growth (as feed additives). Over the last 10 years, the discussion on the role of antibiotics used as additives in livestock feed ran high.

One can not deny that resistance to antibiotics of gut flora increased significantly in animals and humans. In Denmark, vancomycin-resistant enterococci of broiler flocks were isolated from faeces in 11 of 12 farms (92 %) were avoparcin was used as a feed additive. In the non-avoparcin group, only 2 out of 12 farms (17 %) yielded vancomycin-resistant isolates (Bager et al., 1997). The prevalence of resistant *Escherichia coli* in Danish pigs increased between 1970-71 and 1987-88 from 63 % to 100 % (Aalbaeck et al., 1991). Likewise, the widespread use of antibiotics in human medicine encouraged the rapid emergence of resistant bacteria, that become a part of the commensal flora of healthy individuals. The prevalence of vancomycin-resistant enterococci in Europe rose from 0 % to 20 % between 1986 and 1992 (Corpet, 1996). In Germany, the occurrence of ampicillin-resistant *E. coli* isolated from intensive care units is about 50 to 60 %. For general wards, ampicillin-resistant *E. coli* represents 30 % (Shah et al., 1993).

The emergence of an increasing number of resistant bacteria raises concern about their spread in the environment. The possible routes of transmission of antibiotic-resistant bacteria to humans are presented in figure 1. Direct contact with faeces of an infected animal or person, as well as indirect contact with contaminated soil, water or food can result in the transmission of potentially pathogenic bacteria (Donnelly et al., 1997). Most routes of transmission are well-documented and show that infections originate due to a lack of hygiene while handling animals or animal products.

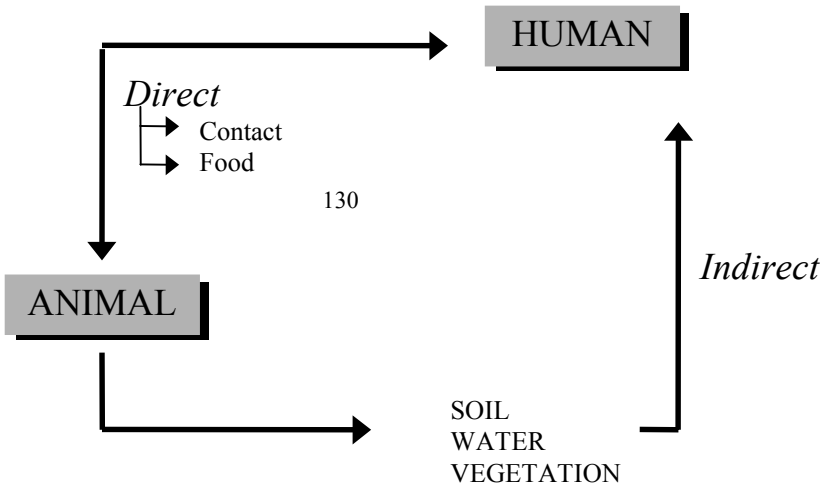

Figure 1
Possible routes of transmission of antibiotic resistant bacteria to humans.

Little is known about the role of the soil as a transport medium for resistant bacteria after waste disposal on fields. The survival of bacteria is influenced by many parameters, such as temperature, soil water content, pH, soil type, presence of other micro-organisms, etc. *E. coli* and *Enterococcus* spp are not common soil bacteria but are normal inhabitants of the intestinal tract of humans and animals, which can cause nosocomial outbreaks in immunocompromised patients. The optimum temperature for growth is about 37 °C, with a pH range from 6 to 7. After excretion, these potentially pathogenic bacteria can survive well in slurry, which is a mixture of faeces, urine and water. Land spreading of these slurries so seems an obvious factor in the dispersion of antibiotic resistant organisms.

We studied the survival of *E. coli* and *Enterococcus* spp from pig manure in soil at different temperatures, moisture contents and manure application rates.

2. Materials and methods

In a first experiment the influence of temperature and moisture content was studied by incubating soil cores with 5 different treatments : (Temperature = T, Moisture Content = MC, Water Holding Capacity = WHC)

1. T = 5 °C, MC = 100 % WHC
2. T = 5 °C, MC = 80 % WHC
3. T = 5 °C, MC = 40 % WHC
4. T = 15°C, MC = 100 % WHC
5. T = 25 °C, MC = 100 % WHC

For all treatments, a loamy textured soil (570 g) was used, homogeneously mixed with pig manure (60 g) which contained about 10^6 cfu/g of *E. coli* and 10^6 to 10^7 cfu/g of *Enterococcus* spp. As a tracer we added 1 ml of a solution containing 10^8 cfu/ml of temocillin-resistant *E. coli*.

With the same soil and manure, a second series of soil cores was incubated, where pig manure was added on the soil surface instead of incorporating it into the soil. Bacterial counts (for the three species) were determined by dilution plating at day 1, 5, 8, 12, 19, 26, 40, 54 and 68.

At the time of analysis, 1 g of topsoil and 1 g of subsoil (about 8 cm depth) were both diluted in 10 ml of physiological water (OXOID, BR 053 G), homogenized by vortex and inoculated on PTX agar (OXOID CM 943 B) (for all *E. coli*), MacConkey agar (OXOID CM 115) containing temocillin (for temocillin-resistant *E. coli*) and Enterococcosel agar (BBL 12205) (for enterococci). Plates were incubated at 37 °C for 24 or 48 h. Enrichment experiments were done for samples in which no bacteria could be detected by direct plating. Therefor, 10 g, 1 g and 0.1 g of soil were diluted in 10 ml of Tryptone Soya Broth (OXOID CM 129), incubated for 24 hours and inoculated on PTX, MacConkey and Enterococcosel.

The survival of *E. coli* and *Enterococcus* spp under different manure application rates was studied for a shorter period of time in a third experiment. A loamy textured soil (114 g) was used for all treatments, homogeneously mixed with a dose of 12 g (410 ton/ha), 6 g (205 ton/ha) or 3 g (102 ton/ha) of pig manure. As a tracer, 0.25 ml (containing 1.10^8 cfu/ml) of a temocillin-resistant *E.* coli was added to each treatment. Bacterial counts were determined on day 1, 5, 8 and 12. One g of soil was diluted in 10 ml of physiological water, shaken and inoculated on PTX agar, MacConkey agar containing temocillin, and Enterococcosel agar. Plates were incubated at 37 °C for 24 or 48 h.

3. Results and Discussion

Preliminary results indicate that during the first 68 days, *E. coli* and *Enterococcus* spp both survive very well in the soil. *Enterococcus* spp survive better than *E. coli*. Bacterial counts of *Enterococcus* spp decrease with 1 logarithmic unit (LU) after 68 days, whereas counts of *E. coli* decrease with 1.5 to 2 LU. Bacteria in the topsoil show a prolonged survival compared to bacteria in subsoil.

The influence of moisture content at 5 °C on bacterial growth is presented in figure 2. *E. coli*, as well as *Enterococcus* spp survive well at levels above detection limit.

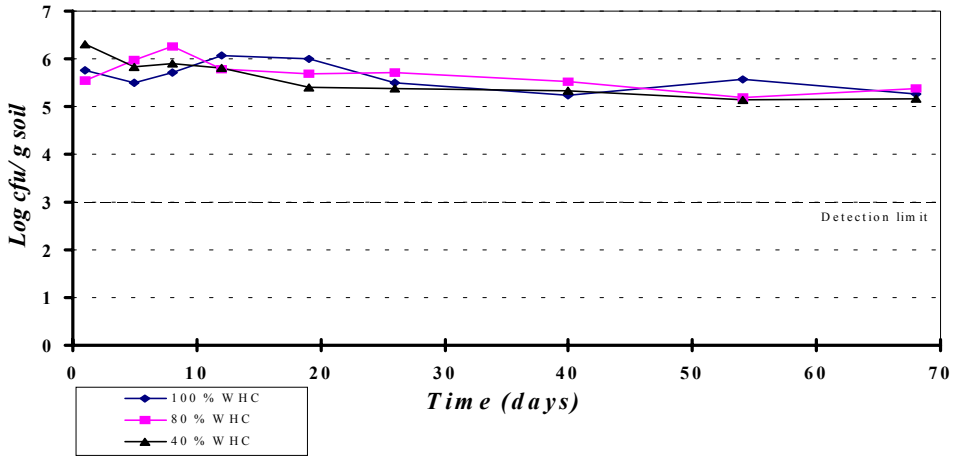

Figure 2a
Survival of Enterococcus spp at different moisture levels (topsoil)

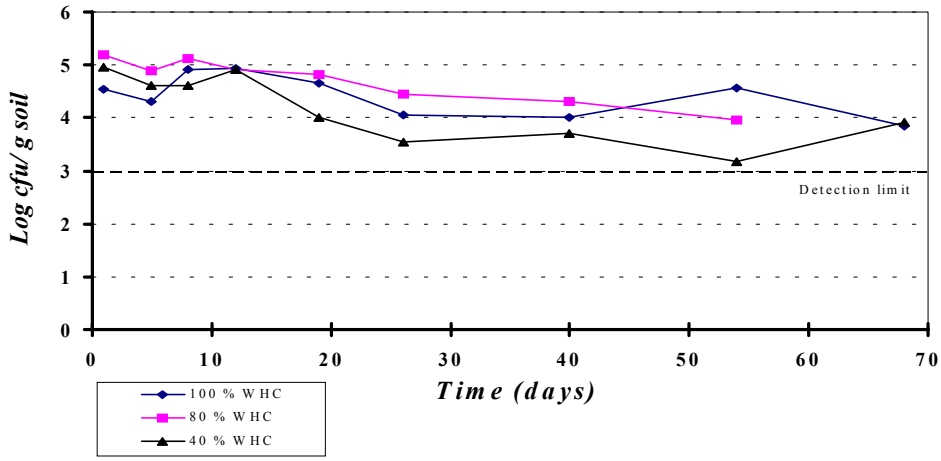

Figure 2b
Survival of *E. coli* at different moisture levels (topsoil)

Increasing the temperature causes a decreased survival of both species, as shown in figure 3. Sixty-eight days after inoculation (dai), *E. coli* survive well at 5 °C and show a bacterial count of about 10^3 cfu/g soil at 15 °C. At 25 °C, detection is only possible until 40 to 50 dai. Enrichment of the soil however indicates that *E. coli* did not completely vanish. For enterococci, survival is only affected at a temperature of 25 °C, where the number of enterococci decreases as low as the detection limit. A decrease in survival at higher temperatures probably results from an increased competition with soil micro-organisms and predation by protozoa.

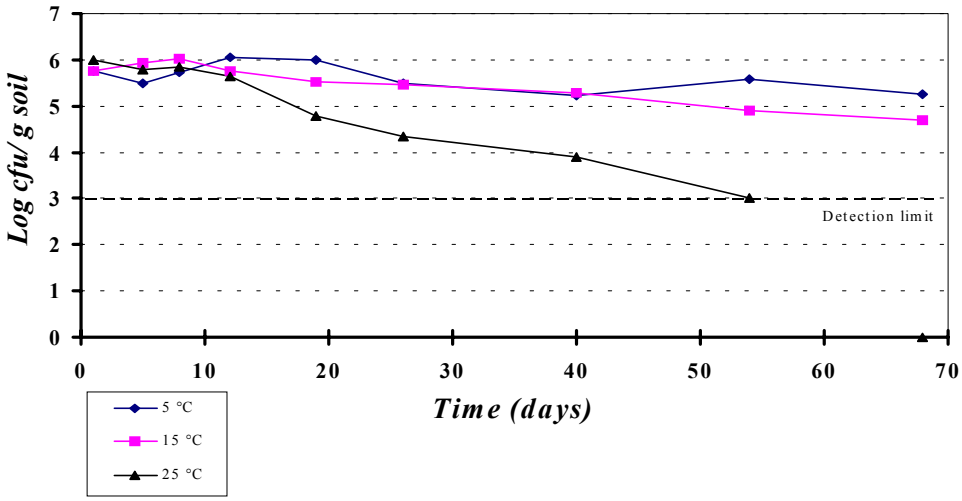

Figure 3a
Survival of Enterococcus spp at different temperature levels (topsoil)

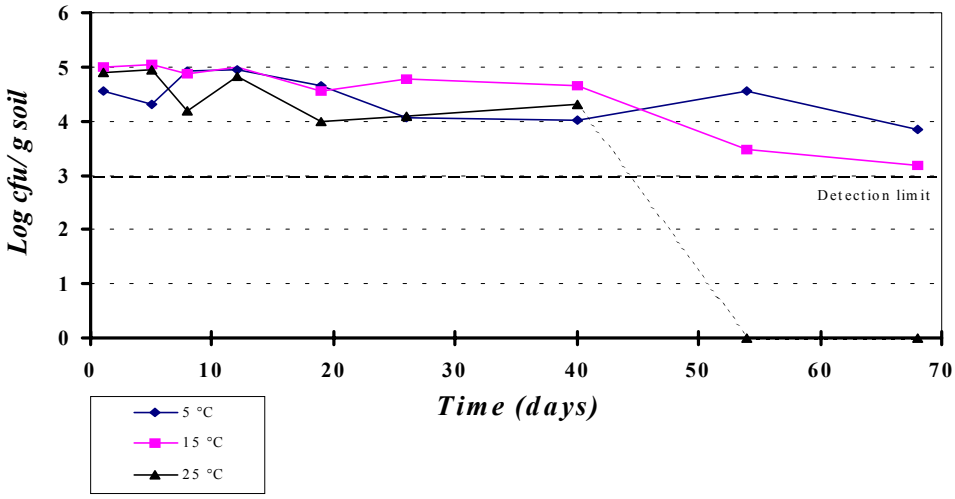

Figure 3b
Survival of E. coli at different temperature levels (topsoil)

When manure is applied on the surface of the soil core instead of incorporating it into the soil, almost no infiltration of pathogens into deeper layers occurs. In the manure layer, a good survival of both species is observed (figure 4).

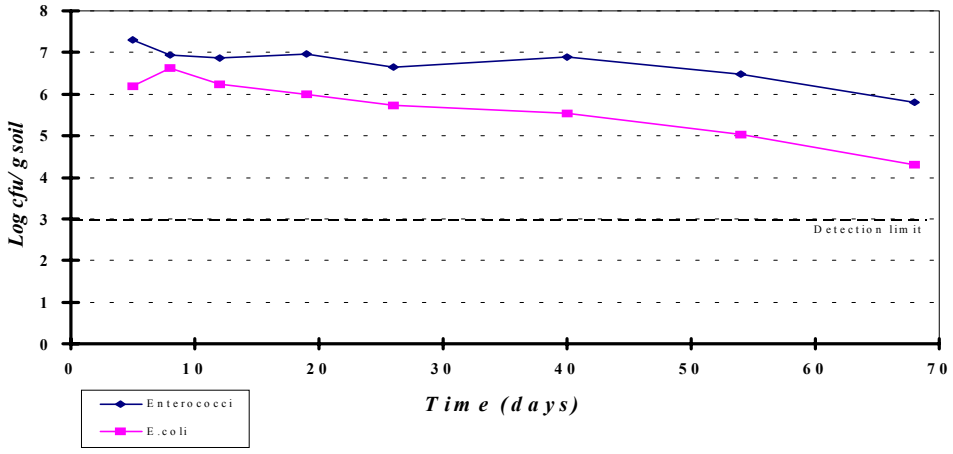

Figure 4
Survival of Enterococcus spp and E. coli in manure on the soil surface

Figure 5 presents the survival of *E. coli* and *Enterococcus* spp at different manure application rates. Since the manure was enriched with an amount of *E. coli,* the initial number of *E. coli* is the same for all treatments.

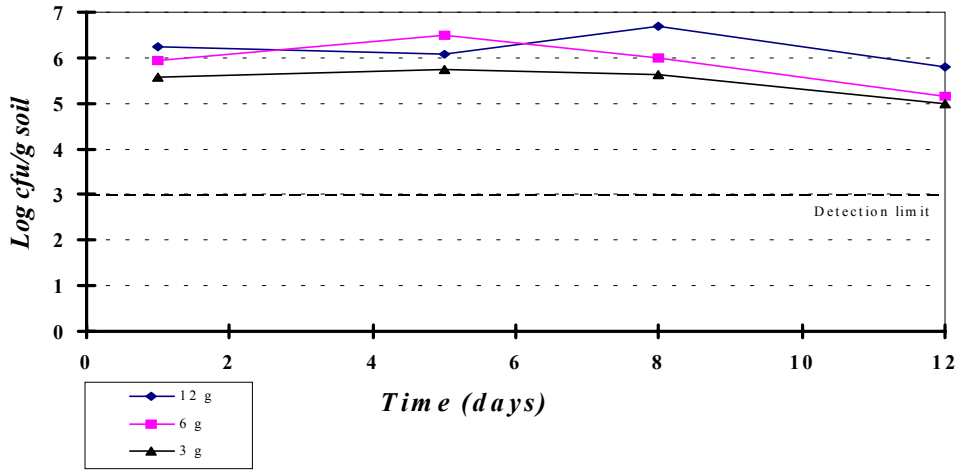

Figure 5a
Survival of Enterococcus spp at different manure application rates

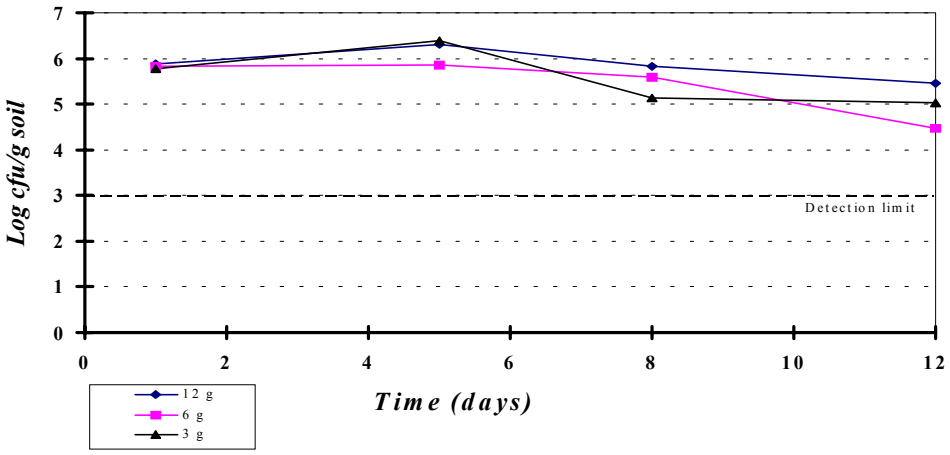

Figure 5b
Survival of E. coli at different manure application rates

Statistical analysis (2-factor ANOVA) of the results indicates that application rate as well as time affect the survival of both species. The main results are presented in table 1.

a. Factor time : For enterococci, survival does not differ during the first 8 days, but at day 12, a different bacterial number was observed. For *E. coli,* sampling data of day 1 and 5, day 5 and 8, and day 8 and 12 do not differ. This means that growth on day 1 differs only significantly from day 8 and 12, even as growth on day 5 differs only from day 12. In other words one can state that over a longer time period, the survival of *E. coli* and *Enterococcus* spp decreases significantly in comparison with the initial bacterial count.

b. Factor dose : Growth of enterococci is significantly different at each manure dose, while for *E. coli* growth is the same at a dose of 6 g and 3 g.

Factor TIME				Factor DOSE		
	Enterococci	E. coli			Enterococci	E. coli
Day 1	A	A		12 g	A	A
	A	A				
Day 5	A	A	B	6 g	B	B
	A		B			B
Day 8	A	C	B	3 g	C	B
		C				
Day 12	B	C				

Table 1
Main results of 2-factor ANOVA

Factor levels with the same letter are not significantly different

4. Conclusion

4.1. Preliminary results of survival experiments of fecal bacteria in soil indicate that both species survive well over a period of 68 days. *Enterococcus* spp survive better than *E. coli* and bacteria in topsoil show a prolonged survival compared to bacteria in subsoil.

4.2. Moisture content does not affect the survival of either species. Increasing temperature levels cause a decreasing survival of *E. coli* at 15 °C and 25 °C, of *Enterococcus* spp only at 25 °C. Both species survive well in a manure layer applied on the soil surface. Also the manure application rate has an influence on bacterial growth.

5. Acknowledgments

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6. References

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