Monitoring Pathogens on Pasture Derived from Treated Wastewater and Biosolids Applications

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ABSTRACT

Treated municipal wastewater and biosolids are increasingly being applied to pasture as a means of developing long term sustainable wastewater solutions. At the time wastewater and biosolids are applied to pasture human pathogens may be present and can potentially persist or even multiply. The use of that pasture and fate of the pathogens will vary for each location and management system. Pasture can be consumed by stock insitu or it can be made into silage or baleage and then consumed by stock; and then for both scenarios humans consume the stock or products from that stock. The risk is generally considered low but what assurances are in place?

The need to address the fate of pathogens is primarily linked to public health risk. Stock health risk is considered low but there are isolated examples overseas of animals being affected by human pathogens. The impact of the varied environments on pathogen populations is unclear, and in many cases inconsistent. Climatic conditions, animal digestion, baleage fermentation and the physical circumstances from soil, crop, baleage, stock and products for sale can all influence pathogen population.

Monitoring of the potential pathogen population from wastewater sources to human consumption requires decisions about:

- What pathogens are of concern;
- When and where should pathogens be monitored;
- What are the limits;
- Can the pathogens be analysed; and
- What level of monitoring is cost effective to manage risks.

This paper describes considerations when evaluating potential pathogen contamination and the level of risk associated to products that are part of the wastewater and biosolid treated pasture pathway. Conclusions are made to how the pathogen risk can be monitored currently and what future investigation is needed to establish increased accuracy.

KEY WORDS

Wastewater, biosolids, pathogens, baleage, monitoring.

INTRODUCTION

Typically consent conditions do not require pathogens that can potentially infect humans to be measured once wastewater and biosolids are applied to land. Consent conditions may provide a two to fourteen day withholding period after applications to land before any animals are grazed. Pathogens are often cited as low risk for further transmission after land application, therefore of little concern. The reality of each circumstance when municipal wastewater or biosolids are applied to land is that the pathogen level is unknown. If people are consuming products after the application it is important to provide some assurances the

risk is low. It is also important for persons handling the discharged material to recognise the associated risks.

A variety of different environments and land uses can receive land applications of treated wastewater. Each of these environments can be exposed to pathogens but the risk needs to be managed when wastewater is applied to pasture in particular. As noted above the risk is small when wastewater is applied to pasture, but there is potential for stock to become infected with pathogens that affect human health. The level of risk is unclear for humans consuming the animal products.

This paper concentrates on monitoring pathogens when wastewater is applied to pasture. The objective is to avoid the animals on that pasture being infected. If the animals are not at risk of infection consequently there is no risk for humans being infected in consumption of the associated animal products. The paper does not discuss the options for wastewater applications to vegetable or fruit production that results in direct human consumption.

This paper introduces the situation by describing the migration of pathogens from the applied material, as treated wastewater or biosolids, to the pasture and on to human consumption via livestock. The pathogens of concern are then identified and situations where contamination can occur. Finally a monitoring approach is outlined followed by remedial action if pathogens are detected.

Reference to pathogens in this paper relates to those that can infect humans and referred to as human pathogens. Although industrial wastewater can contain a municipal component, the focus is on municipal wastewater because this is the primary source of pathogens of concern to human health; and consequently the use of the term wastewater in this paper refers to treated municipal wastewater.

MIGRATION PATHWAY

Pathogens that end up being ingested by humans follow a pathway after land application. Understanding this pathway can provide guidance to minimise the potential for consumption of pathogens and illness. This pathway is summarised in Figure 1 below.

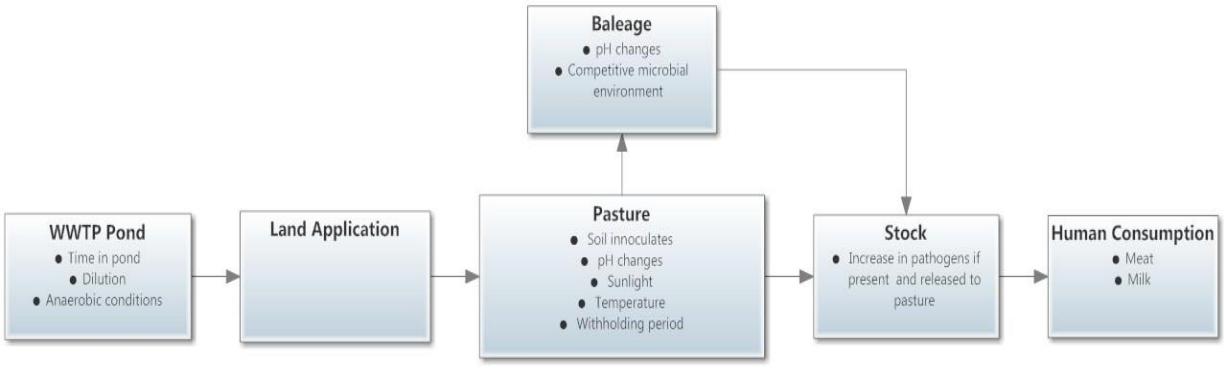

Figure 1: Pathogen Migration Pathway

There are a number of steps and actions along this pathway that can impact on and influence pathogens reaching human receptors.

WWTP - In the WWTP two key factors decrease pathogen numbers, dilution and dieoff. Dilution - There may be peaks in concentration of human pathogens entering the

treatment plant at one time but these are diluted by the large volume of water held in the ponds/treatment system. This means that when applied the human pathogen population will be an average and not influenced by spikes in the influent. Dieoff - The time spent in the treatment ponds allows for pathogen numbers to reduce over time. This can be particularly influenced by pond conditions, especially the presence of anaerobic conditions.

Application - Pathogen numbers may be present in the wastewater or biosolids at the time of land application. It is important for persons handling the material when applying it to land to take due health and safety precautions.

Neighbours can be concerned about potential contamination when land applications are carried out. Runoff into rivers and seepage into groundwater of wastewater from the land treatment areas is negligible. The risk occurs when pathogens are transferred into the groundwater or the river and then the water is used for the production of vegetables. Structures and facilities should be put in place to avoid contamination outside the application area. Precautions to avoid runoff and drainage into groundwater include precise timing of discharge to land to limit drainage, wipe off drains to collect any runoff from border strips and sufficient buffers that separate land application areas. Flooding is one time when runoff cannot be avoided, however the dilution from flooding is likely to reduce pathogen levels further to negligible populations.

Pasture - Application to the soil environment is a key part of pathogen amelioration. Time spent on land provides opportunities for environmental factors to reduce pathogen numbers. This includes natural soil inoculates, changes in pH, sunlight and temperature.

The withholding period before stock are grazed or baleage/silage is harvested can vary. This is dependent on factors that influence pathogen populations in the pastural environment. The length of the withholiding period will be the focus of the monitoring programme discussed below.

Baleage -The maturation of baleage creates a competitive microbial environment and pH changes are expected to reduce human pathogen numbers. The development of moulds resulting from poor storage will make the baleage unsaleable and is not part of this discussion.

Stock - The nature and concentration of human pathogens that affect stock are a grey area. Human pathogens can all be passed to cows but it isn't known if it makes them sick (personal communication Jacqui Horswell, ESR 2013). There are international examples of stock having symptoms that are linked to human pathogens (Barton & Craven 1980; Fong & Lipp 2005). There is also a risk that human pathogens from the wastewater develop to a strain that has shifted from being infectious to humans to infectious to stock. There is little information to qualify any examples of this to date.

Pathogen numbers can increase in animal faeces, which act as an incubator that releases a greater number into the environment. This highlights the benefit of reducing human pathogen numbers before stock are involved.

It is assumed that most farmers will drench their stock that will limit some potential disease organisms, particularly helminth ova. Despite the use of drenches, testing is still applicable. The risk may be lowered but still be present and there is always a possibility that drenching hasn't been carried out, has inadequately been carried out or resistance has established.

Humans - The final stage of the migration pathway is the saleable products from that livestock. The chance of humans being infected from meat and milk from stock that have consumed pasture from wastewater irrigated or biosolids applied land is minor. The pathogen has a long pathway of treatment that reduces the populations as identified in Figure 1.

The migration pathway highlights factors that will reduce pathogen numbers. These include dilution, time without a host, temperature, pH changes and microbial competition. It also highlights the points of potential risk, particularly for the persons applying wastewater and biosolids to land and increased populations in stock faeces, but from here it is difficult to identify where to measure the pathogens to create meaningful data, what pathogens to be concerned about, and how to create factors that will reduce risk of contamination.

HUMAN PATHOGENS IN MUNICIPAL WASTEWATER AND BIOSOLIDS

The potential for risks associated with exposure to pathogens should include consideration of three components:

- Source and type of contaminant;
- Migration pathway (discussed above); and
- Receptors

If one of these components is absent the risk is greatly reduced.

Source and type of contaminant

The quality of wastewater entering a municipal WWTP typically includes effluent from:

- Residual domestic water;
- Urban storm runoff; and
- Industrial waste.

The main source of human pathogens is from the residual domestic water.

The pathogens of concern that are selected here are based on experience from an existing council operations, analytical laboratories, research of human waste products (Guan & Holley, 2003; Smith et al; 2005; Wang et al. 2004; Wery et al, 2008) and the Biosolids Guidelines (NZWWA 2003).

The production of Biosolids Guidelines included extensive review of pathogens in New Zealand conditions and concluded that the following pathogens should be tested:

- *E.coli;*
- Campylobacter;
- Salmonella;
- Enteric virus; and
- Helminth ova.

In addition to these, further enquiry regarding wastewater to the above mentioned sources indicates that Listeria monocytogenes should also be tested for. Cryptosporidium and Giardia are known pathogens in New Zealand but there are no reliable tests for biosolids

and it is assumed they are as unreliable for wastewater. When there are reliable tests these need to be added.

Receptors
Pathogens can be transferred by four means to the receptors:

- Aerosols;
- Food products;
- Direct contact; and
- Water bodies.

The primary receptors are persons in direct contact with the wastewater and biosolids when they are applied to land, and then the livestock grazing the pasture. As described in the migration pathway the risk for receptors is greatly reduced as the migration progresses. The objective here is to eliminate the livestock as a receptor by reducing human pathogens before they consume treated pasture or baleage.

MONITORING PATHOGENS

Monitoring for pathogens aims to determine the human pathogen population. The information then enables decisions when to introduce livestock onto the pasture or to consume baleage. The pathogen population needs to be low enough to significantly reduce livestock as a receptor and avoid any chance of pathogen population increases.

Livestock are recognised as a potential incubator for the pathogens resulting in increased populations released onto pasture through faecal material. For this reason the human pathogen population needs to be below detection, or at a nominally low level, to create confidence the migration pathway is restricted.

The development of a monitoring programme requires the following to be determined:

- Pathogens to monitor;
- Timing the sampling; and
- Pathogen thresholds.

The monitoring programme needs to provide meaningful information and keep the number of sampling occasions to a minimum, to avoid unnecessary costs.

Pathogens to monitor
The human pathogens of concern are identified above. Typically *E.coli* is used as an indicator for the presence of other pathogens. It is understood that *E.coli* does not provide a direct correlation but can indicate if further testing should be carried out. If the *E.coli* threshold is reached a wider suite of pathogens should to be monitored.

Although *E.coli* can provide some indication to the pathogens of concern, it is unlikely to indicate if any *Helminth* ova are present. *Helminth* are most likely to be of concern with the application of biosolids rather than wastewater because of its inclination to sink and accumulate in the solids within the treatment process.

Timing monitoring
Table 1 indicates the potential timing and type of material to be sampled. Sampling for pathogens is sequential following the migration pathway. No detection of pathogens in the wastewater or biosolids samples eliminates further pathogen sampling on pasture and

baleage. If pathogens are present then testing of pasture is required. If pathogens are present on the pasture then baleage will also require testing for pathogens.

It is suggested that only *E.coli* be tested in the wastewater or biosolids to be applied to land. If a nominated *E.coli* threshold is met the wider suite of pathogens should be tested on pasture.

The timing of the *Helminth* ova analysis is uncertain and may require a variety of sampling periods to determine the best indication when the risk is low. This could be particular to the treatment plant/process. To restrict *Helminth* tests to just biosolids is yet another question that may require further testing to determine if wastewater also requires analysis.

Table 1: Sampling Pathogens from Municipal WWTP – location, timing and type of analysis

Material Analysed	**Wastewater & Biosolids**	**Pasture**	**Baleage**
Location of Material and Timing of Sample Collection	Prior to each land application or on a regular routine basis.	Prior to harvest of baleage or prior to grazing **if** the type of pathogen is detected in the wastewater or biosolids. May want to be in a representative area and under conditions which have highest risk, i.e. moist warm and cloudy conditions.	With quality testing only **if** pathogens have been detected in the pasture.

Soil sampling has not been included. There is a small risk that soil can be incorporated into the baleage, but the information from soil sampling for pathogens will be limited by comparison to the other types of samples: wastewater, biosolids, pasture or baleage.

Pathogen Thresholds

For the pathogens of concern, Table 2 lists minimal doses that will cause infections in humans and identifies the type of organism the pathogens are.

Table 2: Minimal Infective Doses in Humans

Pathogen	**Type**	**Minimum infective dose**
[1]Ascaris (worm)	Parasitic worm	1-10 eggs
[1]Salmonella spp.	Bacteria	10,000-10 million
[1]E.coli	Bacteria	1 million -100 million
[1]Campylobacter jejuni	Bacteria	~500
[2] Listeria	Bacteria	10-100 million cfu
[1]Giardia lambilia	Protozoa	10-100 cysts
[3]Enteric virus	Virus	<1 $TCID_{50}$

([1]Bitton 1994; [2]Farber et al., 1996, [3]Yezli & Otter, 2005)

To restrict the migration pathway and avoid livestock increasing populations by the oral-faecal route, it is recommended the threshold for each pathogen in pasture be less than 1, i.e. not detected. However, this number could be subject to debate and dependent on the risks of transmission, as influenced by with-holding periods etc.

The question that still requires determination is what level of *E.coli*, in the first step of testing the wastewater discharge or biosolids, should determine further testing for the wider suite of pathogens on pasture and baleage/silage.

REMEDIAL ACTION

Figure 2 depicts the sampling approach and associated remedial action, as discussed above.

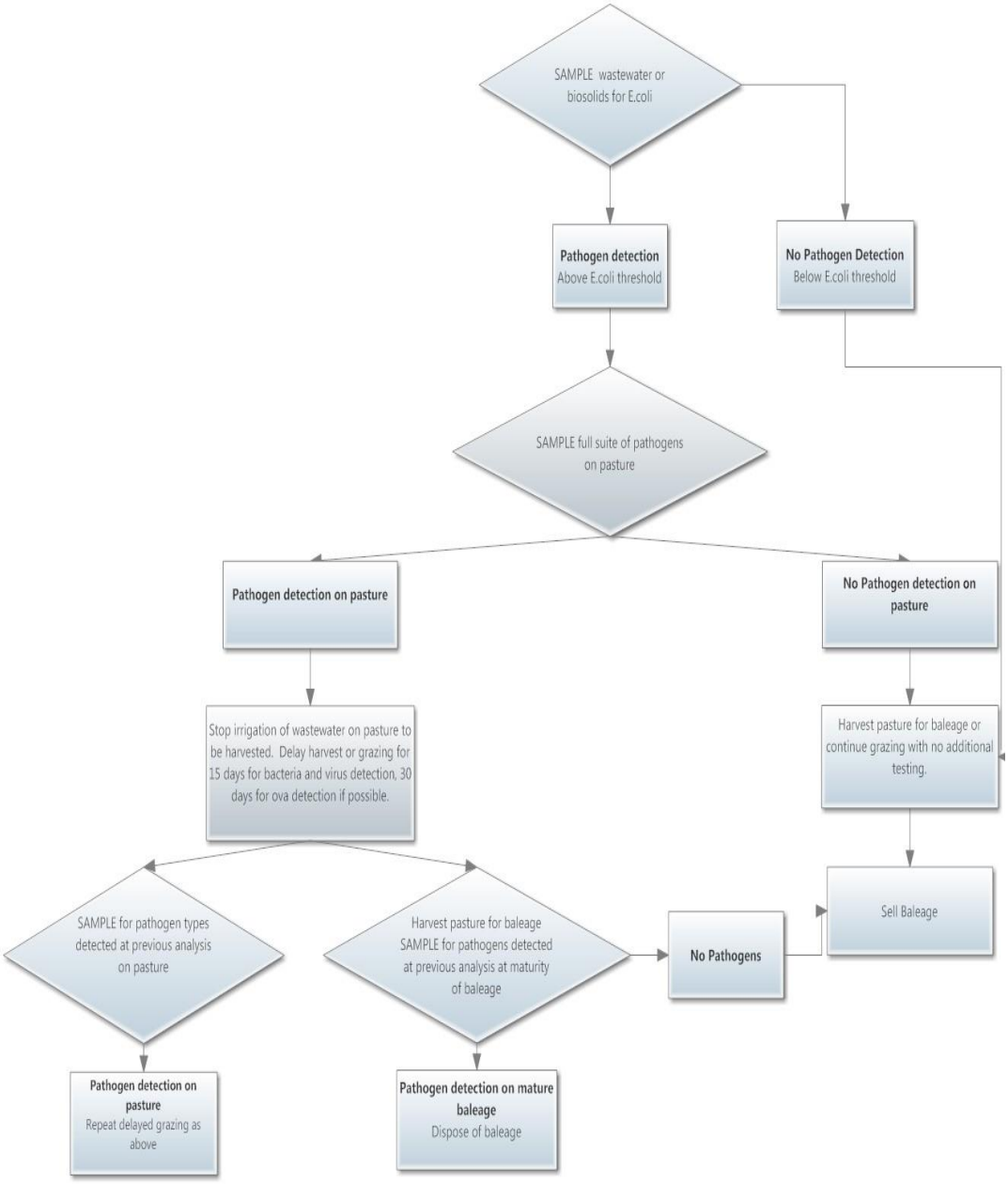

Figure 2: Remedial action on detection of pathogens

CONCLUSION

There is a potential risk when applying treated wastewater to land that pathogens can infect humans. The risk is low but one isolated event could result in negative consequences for land application systems.

The objective of the proposed monitoring of human pathogens is to avoid the opportunity for stock to be infected and to develop a tiered screening approach to avoid all material having to be sampled on an ongoing basis.

The stock are unlikely to become ill from human pathogens but are the link between potential human infection. Reducing the risk for stock infection consequently reduces the risk for human infection.

Monitoring provides evidence that human pathogens have been reduced before potential contamination can occur. However, when a person becomes very unwell it is possible the victim looks for opportunities to pass blame, and therefore traceability of animal products and a definite link to monitoring results is considered essential.

There are unanswered questions that will require determination through trials and further discussion to limit unnecessary analysis and conjecture about risks. The questions are:

- What level of E.coli should provide a trigger for further analysis of a wider suite of human pathogens?
- When should Helminth ova be tested for?

REFERENCES

Barton M. D., Craven J.A. (1980) Listeriosis pathology, bacteriology and serology. Australian Bureau of Animal Health.

Bitton, G. (1994) Wastewater Microbiology. New York: Wiley-Liss, Inc., p. 77-78. and Biocycle, September 1998, p. 6.

Farber, J. M., Ross, W. H., & Harwig, J. (1996) Health risk assessment of Listeria monocytogenes in Canada. International Journal of Food Microbiology, 30(1-2), 145-156.

Fong T.T., Lipp E. K. (2005) Enteric Viruses of Humans and Animals in Aquatic Environments: Health Risks, Detection, and Potential Water Quality Assessment Tools. Microbiol Mol Biol Rev. June; 69(2): 357–371.

Guan T.Y., Holley R.A. (2003) Pathogen survival in swine manure environments and transmission of human enteric illness--a review. Journal of Environmental Quality. Mar-Apr; 32(2):383-92.

NZWWA (2003) Guidelines for the safe application of Biosolids to land in New Zealand. New Zealand Water and Wastes Association. 177pp.

Smith, Jr., J.E., P.D. Millner, W. Jakubowski, N.Goldstein, and R. Rynk, eds. (2005) Contemporary Perspectives on Infectious Disease Agents in Sewage Sludge and Manure. The JG Press.

Wang, H., Magesan G. N. & Bolan, N.S. (2004) An overview of the environmental effects of land application of farm effluents, New Zealand Journal of Agricultural Research, 47(4), 389-403.

Wery N., Lhoutellier C., Ducray F., Delgenes J.P., Godon J.J., (2008) Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR. Water Research. 42 (1–2): 53–62.

Yezli S., Otter, J.A. (2011) Minimum Infective Dose of the Major Human Respiratory and Enteric Viruses Transmitted Through Food and the Environment Food and Environmental Virology. March 2011, 3(1): 1-30