

EVALUATION OF MICROBIAL HEALTH RISKS ASSOCIATED WITH THE REUSE OF SOURCE-SEPARATED HUMAN URINE

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Introduction

Human excreta contain plant nutrients and have traditionally been used for crop fertilisation in many countries. In Japan the recycling of urine and faeces was introduced in the 12th Century and in China human and animal excreta have been composted for thousands of years. Urine is the fraction that contains the major part of the nutrients in domestic wastewater, approximately 80% of the nitrogen, 55% of the phosphorous and 60% of the potassium (Swedish EPA, 1995). At the same time it constitutes less than 1% of the total wastewater volume. Thus it is possible to collect a relatively concentrated fertiliser by separating urine from the wastewater. Faeces contribute a smaller amount of nutrients and involves greater health risks if reused due to the possible presence of enteric pathogens. Human urine does not generally contain pathogens that can be transmitted through the environment.

Microorganisms in urine

In a healthy individual the urine is sterile in the bladder. When transported out of the body different types of dermal bacteria are picked up and freshly excreted urine normally contains <10 000 bacteria per ml (Tortora *et al.* 1992). Pathogens that may be transmitted through urine are rarely sufficiently common to constitute a significant public health problem and are thus not considered to constitute a health risk related to the reuse of human urine in temperate climates. An exception in tropical areas is *Schistosoma haematobium*, which however implies a low risk due to its lifecycle where a freshwater snail is needed as an intermediate host. Furthermore, the inactivation of urinary excreted pathogens in the environment reduces their ability for transmission.

Aim of the study

Source-separation of urine and faeces is possible by using urine-separating (or urine-diverting) toilets, available as simple dry toilets or porcelain flush toilets with divided bowls.

The aim of this study was to investigate and evaluate health risks from infectious diseases related to handling and reuse of source-separated urine in agriculture.

The specific objectives were:

- to determine the faecal contamination that occurs in urine-separating toilets;
- to determine the inactivation rates of different groups of microorganisms in source-separated human urine and relate the inactivation to some of the characteristics of the urine mixture (urine + flushwater);
- to quantify microbial health risks in urine-separating sanitation systems by using Quantitative Microbial Risk Assessment (QMRA).

Faecal cross-contamination

Any faecal cross-contamination that may occur by misplacement of faeces in the urine-separating toilet is regarded as a possible health risk.

The presence of human faeces in urine samples was successfully determined by analysing for faecal sterols. Cross-contamination was evident in 28% of the samples from urine collection tanks. In tanks where the urine was found to be contaminated, it was possible to calculate the amount of faecal matter still in suspension. Using an average value of 4 µg coprostanol per mg faeces, contamination was calculated to vary between 1.6 and 18.5 mg of faeces per l urine mixture with a mean of 9.1 ± 5.6 mg/l.

Analysis of various indicator bacteria implied different degrees of faecal contamination if evaluated according to their normal abundance in faeces, which in further investigations partly could be explained by different growth and survival characteristics. *E. coli* had a rapid inactivation in the urine and faecal streptococci were found to grow within the urine pipes. It was concluded that none of the commonly used indicator bacteria were suitable to quantify faecal cross-contamination in source-separated urine.

Survival of microorganisms in urine

The fate of the enteric pathogens entering the urine tank is of vital importance for the health risks related to the handling and reuse of the urine. To determine the duration and conditions for sufficient storage of the urine mixture before its use as a fertiliser, it was therefore necessary to estimate the survival of various microorganisms in urine as a function of time.

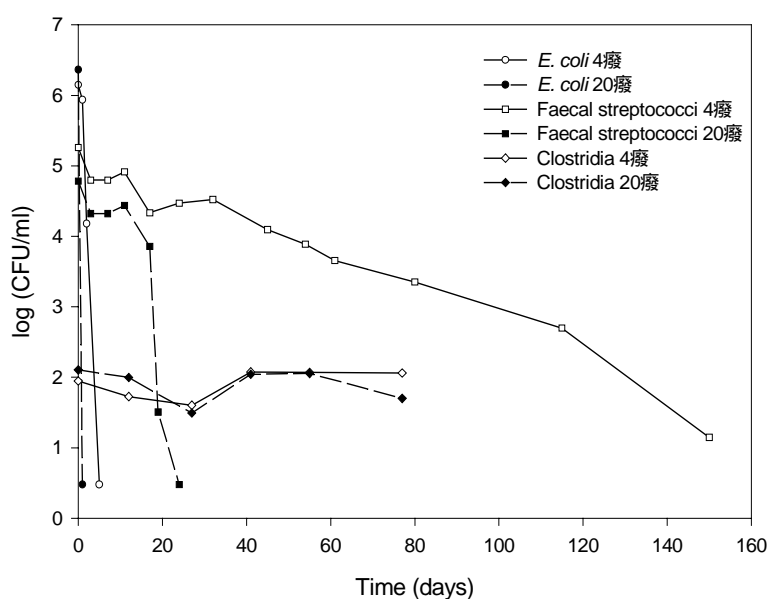


Figure 1. Inactivation of *E. coli*, faecal streptococci and *C. perfringens* spores (clostridia) in source-separated human urine (pH 9) at 4°C and 20°C.

Gram-negative bacteria (e.g. *Salmonella* and *E. coli*), which cause a majority of gastrointestinal infections, were rapidly inactivated (time for 90% reduction, $T_{90} < 5$ days) in source-separated urine at its natural pH-value of 9. Gram-positive faecal streptococci were more persistent with a T_{90} of approximately 30 days at 4°C. Clostridia spore numbers were not reduced at all during 80 days (Figure 1). A lower temperature and a higher dilution involved a longer survival of most bacteria. pH-

values the furthest from neutral had the most negative effect on survival of the organisms. At pH 6 most of the bacteria had a better survival than at pH 9. The reduction of bacteria at high pH-values may be an effect partly of the pH and partly of the presence of ammonia.

In urine mixture at pH 9 and 4°C, oocysts of the protozoa *Cryptosporidium parvum* were inactivated to below the detection limit (<1/300) within 63 days. The T₉₀-value for *Cryptosporidium* was determined at 29 days (Table 1). At 20°C the T₉₀ was estimated at 5 days.

To investigate virus survival rotavirus and *Salmonella typhimurium* phage 28B were chosen as model organisms. In summary, no significant inactivation of either rotavirus or the phage occurred at 5°C during six months of storage, while the mean T₉₀-values at 20°C were estimated at 35 and 71 days, respectively (Table 1). In pH-controls (pH 7), the inactivation of rotavirus was similar to that in urine at both temperatures, whereas no decay of the phage occurred at either 5°C or 20°C. Therefore, rotavirus inactivation appeared to be largely temperature dependent, whereas there was an additional virucidal effect on the phage in urine at 20°C (pH 9).

Table 1. Summarised results from the survival experiments, given as T₉₀-values (time for 90% reduction)

	Gram-negative bacteria	Gram-positive bacteria	<i>C. parvum</i>	Rotavirus	<i>S. typhimurium</i> phage 28B
4°C	1	30	29	172 ^a	1 466 ^a
20°C	1	5	5	35	71

^a survival experiments performed at 5°C

Microbial risk assessment of urine-separating systems

Quantitative Microbial Risk Assessment (QMRA) is a tool used to predict the consequences of potential or actual exposure to infectious microorganisms (Haas *et al.* 1999). Microbial risk assessments were first developed for drinking waters (Regli *et al.* 1991) and have later been applied to practices such as irrigation of crops.

The transmission pathways investigated in the QMRA included accidental ingestion of unstored urine (1 ml); accidental ingestion of stored urine (1 ml); inhalation of aerosols while spreading the urine; and ingestion of crops contaminated by urine. Persons at risk include inhabitants in the housing area; workers handling the urine, including farmers applying the urine to arable land; persons in the surroundings of the field; and persons consuming fertilised crops.

Calculations of the doses ingested were based on the measured faecal contamination, the incidence of infection by *Campylobacter jejuni*, *Cryptosporidium parvum* and rotavirus in the population, the excretion of these pathogens and their inactivation in urine mixture. Finally the risks for infection were calculated by using dose-response models.

Except for rotavirus, calculated risks were below 10⁻³ (1:1 000) for all exposure routes independent of the urine storage time and temperature evaluated. Due to the persistence of rotavirus at low temperatures (≤5°C) and a low infectious dose risks for rotavirus infection were up to 0.56 by ingestion of unstored and stored (4°C) urine. If stored at a higher temperature (20°C) for six months, risk for rotavirus infection decreased to below 10⁻³. The risk for *Campylobacter* infection was negligible (<10⁻¹⁵) except if unstored urine was handled or used for fertilising. *Cryptosporidium*

constituted a lower risk in unstored urine than *Campylobacter* but six months storage at 20°C was needed for risks to be negligible.

The risk from ingestion of contaminated crops will be dependent on the time that passes between fertilisation and harvest of the crop, i.e. consumption, since pathogen inactivation will continue on the crop due to UV-radiation, desiccation etc. In Figure 2, the risks from consumption of crops one to four weeks after fertilising with unstored urine are presented. The risk for bacterial or protozoan infection was $<10^{-5}$ after one week, whereas three weeks were needed for the risk of viral infection to be of the same magnitude.

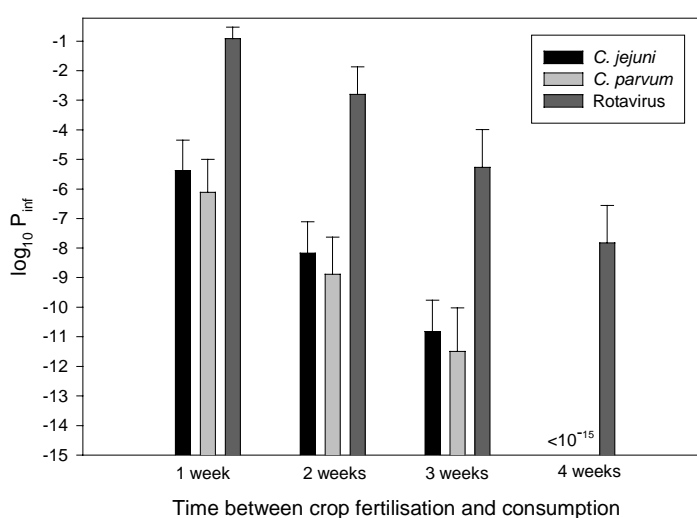


Figure 2. Mean probability of infection by pathogens following ingestion of 100 g crop fertilised with unstored urine with varying time between fertilisation and consumption. Error bars indicate one standard deviation.

Guidelines for the reuse of human urine

Since urine-separating systems are being implemented in Sweden, it was decided to set reuse conditions based on the parameters urine storage time and temperature (Table 2). Guidelines may in this context be seen as recommendations on how to use source-separated urine in agriculture in order to minimise the risks for transmission of infectious diseases and as a part of risk management. Regulatory standards or guidelines have yet to be determined by the agency responsible.

These guidelines were set based on the inactivation of microorganisms in urine and the results from the risk assessment do not imply that the recommendations need to be modified. Under conditions (i.e. regarding temperature, pH and nitrogen concentration) other than those given, the inactivation may be different.

Table 2. Relationship between storage conditions, pathogen content^a of the urine mixture and recommended crop for larger systems^b. It is assumed that the urine mixture has at least pH 8.8 and a nitrogen concentration of at least 1 g/l

Storage temperature	Storage time	Possible pathogens in the urine mixture	Recommended crops
4°C	≥1 month	viruses, protozoa	food and fodder crops that are to be processed
4°C	≥6 months	viruses	food crops that are to be processed, fodder crops ^c
20°C	≥1 month	viruses	food crops that are to be processed, fodder crops ^c
20°C	≥6 months	probably none	all crops ^d

^a Gram-positive bacteria and spore-forming bacteria are not included.

^b A larger system in this case is a system where the urine mixture is used to fertilise crops that will be consumed

by individuals other than members of the household from which the urine was collected.

^c Not grasslands for production of fodder. Use of straw is also discouraged.

^d For food crops that are consumed raw it is recommended that the urine be applied at least one month before harvesting and that it be incorporated into the ground if the edible parts grow above the soil surface.

Future perspectives

Whether urine-separation and the reuse of urine can be recommended depends on whether the associated health risks are considered to be acceptable. These risks can be balanced against benefits like the fertiliser value of human urine. Higher risks from reuse of waste products may be acceptable in areas where enteric disease is endemic and where it is more often transmitted through poor hygiene and sanitation (Blumenthal *et al.* 2000). In areas where food is scarce, benefits from larger harvests may reduce other risks such as malnutrition, which otherwise causes immunosuppression and makes the individual more susceptible to infections. By following suggested recommendations for storage and reuse, which are dependent on the type of crop to be fertilised, it is possible to significantly decrease the risk for infections. Urine-separation and the reuse of human urine are thus appropriate parts of a sustainable future regarding sanitation.

References

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