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University of Veterinary Medicine
Research Institute of Veterinary Medicine
Hlinkova 1/A
040 01 Košice
Slovak Republic

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

C. SCHÖNNING

Department of Parasitology, Mycology and Environmental Microbiology, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden, tel: +468 4572449, fax: +468 318450, e-mail: caroline.schonning@smi.ki.SE

ABSTRACT

Source-separation of urine and faeces is possible by using urine-separating toilets with divided bowls. The risk for transmission of disease when handling and reusing the urine as a fertiliser in agriculture is largely dependent on the cross-contamination by faeces and the survival of faecal pathogens entering the collected urine. Cross-contamination as analysed by faecal sterols was evident in 28% of the samples from urine collection tanks, and in these quantified to a mean (±SD) of 9.1 ± 5.6 mg faeces per litre urine mixture. Gram-negative bacteria (e.g. *Salmonella* and *E. coli*) were rapidly inactivated (T_{90} <5 days) in source-separated urine at its natural pH-value of 9. Faecal streptococci and *Cryptosporidium* oocysts were more persistent whereas clostridia spores, rotavirus and a bacteriophage were not inactivated in urine at low temperatures (4-5°C). By using Quantitative Microbial Risk Assessment (QMRA), the risks for bacterial and protozoan infections were calculated to be $<10^{-3}$ for all exposure routes independent of the urine storage time and temperature. The risk for viral infection was higher. However, 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.

Key-words: urine, faecal contamination, faecal sterols, microbial survival, QMRA, guidelines.

INTRODUCTION

Human excreta contain plant nutrients and have traditionally been used for crop fertilisation in many countries. 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.

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.

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.

MATERIAL AND METHODS

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 determined by analysing samples from urine collection tanks for faecal sterols and faecal indicator bacteria.

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. Various bacteria were added to source-separated human urine and quantified at various time intervals. The same procedure was followed for the protozoa *Cryptosporidium parvum* and to investigate virus survival rotavirus and *Salmonella typhimurium* phage 28B were chosen as model organisms.

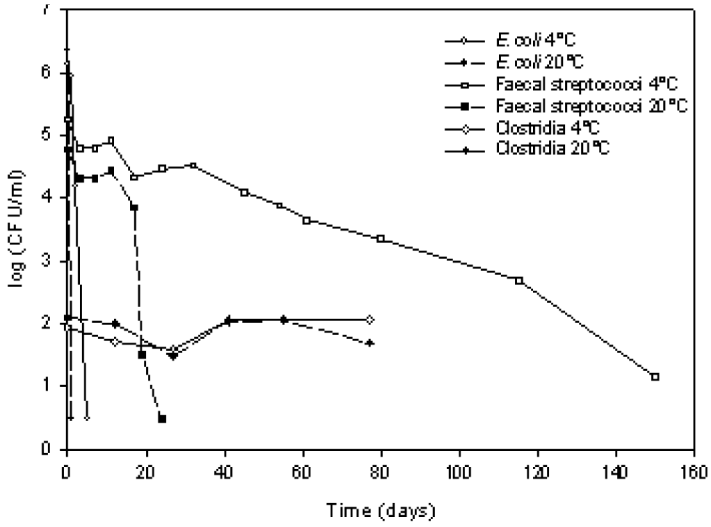
Quantitative Microbial Risk Assessment (QMRA) is a tool used to predict the consequences of potential or actual exposure to infectious microorganisms (Haas et al., 1999). The transmission pathways investigated in the QMRA included accidental ingestion of unstored urine (1 ml); accidental ingestion of 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.

RESULTS AND DISCUSSION

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 $\pm$ 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.

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 (Figure 1). 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.

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.



In urine mixture at pH 9 and 4°C *Cryptosporidium parvum* oocysts were inactivated to below the detection limit (<1/300) within 63 days. The T_{90}-value for *Cryptosporidium* was determined at 29 days (Table 1). At 20°C the T_{90} was estimated at 5 days. No significant inactivation of either rotavirus or the phage occurred at 5°C during six months of storage, while the mean T_{90}-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).

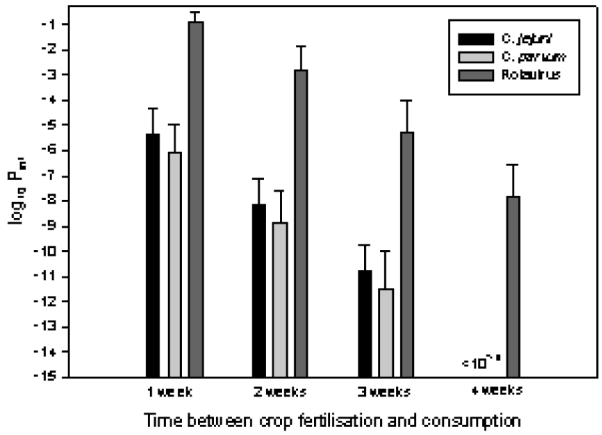
Table 1. Summarised results from the survival experiments, given as T_{90}-values (time for 90% reduction)

	Gram-negative bacteria	Gram-positive bacteria	*C. parvum*	Rotavirus	*S. typhimurium* 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

Except for rotavirus, calculated risks in the QMRA 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.

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.



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.

CONCLUSIONS - 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. By following suggested recommendations for storage and reuse 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.

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.*

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