Critical review of epidemiological evidence of the health effects of wastewater and excreta use in agriculture

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## Glossary of epidemiological terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attributable risk</td>
<td>Is a measure of association that indicates on an absolute scale how much greater the frequency of disease is in the exposed group compared with the unexposed, assuming that the relationship between exposure and disease is causal.</td>
</tr>
<tr>
<td>Attributable risk fraction</td>
<td>The proportion of disease among the exposed group that is attributable to the exposure.</td>
</tr>
<tr>
<td>Confounding</td>
<td>Confounding occurs when an estimate of the association between an exposure and the disease is mixed up with the real effect of another exposure factor on the same disease – the two exposures being correlated. This variable is called a confounder or confounding variable. The estimate of the association that arises is biased.</td>
</tr>
<tr>
<td>Confounding variable/factor</td>
<td>A confounder is a variable that is associated with the exposure under study and is a risk factor for the disease it its own right.</td>
</tr>
<tr>
<td>Cross-sectional study</td>
<td>A study in which information on the putative risk factors and the putative outcomes are measured simultaneously at one point in time.</td>
</tr>
<tr>
<td>Geo-helminths</td>
<td>Soil transmitted helminth infections such as <em>Ascaris</em> and hookworm.</td>
</tr>
<tr>
<td>Odds of disease</td>
<td>The ratio of the probability of getting the disease to the probability of not getting the disease during a given time period.</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>A measure of relative risk. In a cohort study it is given by the odds of disease in the exposed divided by the odds of disease in the unexposed. In a case-control study it is given by the odds of exposure in the cases divided by the odds of exposure in the controls. Results of logistic regression analysis are often reported as odds ratios. The ‘no effect’ level is an odds ratio of 1.</td>
</tr>
<tr>
<td>Prospective cohort study</td>
<td>A study in which a group of persons exposed and a group of persons unexposed to a potential risk factor are followed up in time and the incidence of the outcome in one group compared with the incidence in the other.</td>
</tr>
<tr>
<td>Population attributable fraction</td>
<td>The proportion of disease in the whole study population that is attributable to the exposure (and potentially could be eliminated if the exposure were eliminated). This is the preventable fraction.</td>
</tr>
<tr>
<td>Relative risk</td>
<td>It estimates the magnitude of an association between exposure and disease and indicates the likelihood of developing the disease in those exposed relative to those unexposed. The relative risk is used as a measure of aetiological strength. Three types of measures of relative risk can be calculated: risk ratio, rate ratio and odds ratio.</td>
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</table>
SUMMARY

A critical review of selected epidemiological studies of wastewater and excreta use in agriculture was carried out (including 10 epidemiological studies of consumer risks from wastewater reuse, 25 studies of risks to farm workers and populations living nearby wastewater irrigated fields, and 7 studies of the effect of exposure to excreta or nightsoil used as fertilizer). This enabled the quantification of the risks of gastro-intestinal infections from exposure to untreated wastewater or excreta and provided measures of the effect of wastewater and excreta treatment in reducing such risks. Critical review of the studies showed that methodological problems influenced the validity of the data from some studies. A sub-set of analytical epidemiological studies were selected which included the following features: well-defined exposure and disease, risk estimates calculated after allowance for confounding factors, statistical testing of associations between exposure and disease, and evidence of causality (where available). These were used as a basis for estimating threshold levels below which no excess infection in the exposed population could be expected. Further information on the risks of infection attributable to the exposure, and in particular the proportion of disease in the study population attributable to exposure (and therefore potentially preventable through improvement in wastewater quality), was used to inform proposals on appropriate microbiological guidelines for wastewater reuse in agriculture. However, as yet, the guidelines are not adjusted to take into account local circumstances and concepts of acceptable risk.

For unrestricted irrigation, there was evidence to suggest that the use of untreated wastewater to irrigate vegetables led to increased helminth infection (mainly *Ascaris lumbricoides* infection), bacterial infections (typhoid, cholera, *Helicobacter pylori*) and symptomatic diarrhoeal disease in consumers. When wastewater was partially-treated, there was evidence that the risk of enteric infections (bacterial and viral origin) was still significant when consumers ate some types of uncooked vegetables irrigated by water exceeding WHO guideline of 1000 FC/100ml by a factor of ten. There is no evidence supporting any need to reduce the guideline level below ≤1000 FC/100ml in such circumstances. There is also no epidemiological evidence to indicate the threshold level when wastewater use is introduced to a naïve population. However, when microbiological and risk assessment studies were also taken into account there some evidence to suggest that the *faecal coliform* guideline of ≤1000 FC/100ml would not result in increased infection in exposed groups beyond acceptable limits. Wastewater treatment markedly reduced the risk of helminth infections (particularly *Ascaris* infection) related to consumption of wastewater irrigated crops but the extent of treatment needed to remove the risk could not be fully quantified. In hot and dry conditions, where vegetables are grown commercially and not eaten by local populations prior to sale in shops or markets, a guideline of ≤1 nematode egg per litre may be adequate. However, there was epidemiological evidence that treating the wastewater to the *nematode egg guideline* level of ≤1 nematode egg per litre may not be sufficient in an area where flood irrigation is used and field vegetables are eaten by the local population. The guideline may need to be revised to ≤0.1 egg per litre where those conditions apply, and where children of farm workers are exposed to the wastewater (see below).

For restricted irrigation, studies of the risks of enteric viral and bacterial infections related to use of treated wastewater (both from serological studies and studies of reported enteric infections) suggested that when sprinkler irrigation was used and the population was exposed to wastewater aerosols, there was an increased risk of infection when the quality of the wastewater was 10⁶ TC/100ml but no increased risk of infection when the quality of the wastewater was 10³-10⁴ FC/100ml. Studies of the risks of symptomatic diarrhoeal disease and enteric viral infections related to direct contact with treated wastewater through farm work (adults and children) or play
(children) suggested that when flood or furrow irrigation occurs there was an increased risk of infection in children when the quality of the wastewater was above $10^4$ FC/100ml. For adults, the threshold level for symptomatic diarrhoeal disease was $10^3$ FC/100ml, but the threshold level for transmission of Human Norwalk-like virus/Mexico (as indicated by an increased level of seroresponse) was below $10^4$ FC/100ml where high levels of contact occurred, even in a rural area where there were many other transmission routes for this virus. This was probably not related to an increase in disease in the study population, but could conceivably lead to increased disease in a population in a non-endemic area.

These data support the need for a faecal coliform guideline to protect farm workers, their children, and nearby populations from enteric viral and bacterial infections. The appropriate guideline will depend on which irrigation method is used and who is exposed. For example, if adult farm workers and nearby populations are exposed through spray/sprinkler irrigation, a guideline of $\leq 10^5$ FC/100ml should be adequate. A reduced guideline of $\leq 10^3$ FC per 100ml would be safer where adult farm workers are engaged in flood or furrow irrigation, and where children under 15 years are regularly exposed (through farm work or play). However, a faecal coliform guideline of $\leq 10^5$ FC/100ml could be adopted if effective health promotion measures could be taken to help adult farm workers and children improve hygiene measures post-wastewater contact.

Studies of the risks of Ascaris infection to farm workers and their families related to the use of treated wastewater suggested that there was an increased risk of Ascaris infection in children even when the quality of the wastewater was $\leq 1$ nematode egg per litre. There was evidence to suggest that the threshold may be above 1 nematode egg per litre for adults. The low infectious dose for Ascaris infection, and the persistence of eggs in the environment (such that eggs could accumulate on the soil and result in increasing exposure) could explain the low threshold level. These data suggest that the nematode egg guideline of $\leq 1$ egg per litre is adequate if no children are exposed, but a revised guideline of $\leq 0.1$ egg per litre (equivalent to a no detect level with current sampling techniques) would be safer if children are in contact with the wastewater through irrigation or play. However, if the proportion of the population who are exposed in this way is small, treatment to the level of $\leq 1$ nematode egg per litre could be combined with anthelmintic campaigns to treat children at risk.

Studies of the use of excreta or ‘nightsoil’ to fertilize vegetables indicated that exposure to untreated excreta was associated with an increased prevalence and intensity of Ascaris infection (sometimes associated with an increase in Trichuris trichiura infection), and in some areas also an increased risk of hookworm infection. Effective treatment of excreta before use led to reduced re-infection rates such that a large reduction in the prevalence of Ascaris and hookworm (and to a lesser extent, Trichuris) infections in exposed populations was achieved. The main epidemiological studies assessed the treatment of excreta with chemicals, although heat treatment of excreta is also effective (and can be done on a large scale). There was insufficient evidence of the effectiveness of traditional treatment or composting of faeces in reducing the prevalence of Ascaris and hookworm infection in exposed persons. There has been no significant new data (post 1985) on the effectiveness of excreta treatment in reducing the risks from use of excreta as fertilizer, so no changes are proposed to guidelines for excreta use, other than those implied by changes to the guidelines for wastewater use in agriculture.
1. Introduction.

Reuse of wastewater and excreta in agriculture has a long history, with wastewater being used on ‘sewage farms’ for at least a century and excreta (or nightsoil) being used in the Far East for many centuries. WHO first set guidelines for the safe use of treated wastewater in agriculture in 1973, building upon the standards for reuse set in the State of California in 1968. These were based on the wastewater quality that could be achieved using the treatment technologies available at the time. In the 1970’s and early 1980’s, more literature became available on epidemiological and microbiological studies of waste reuse, which were reviewed through projects set up by World Bank, WHO and UNEP. WHO then revised its guidelines for wastewater and excreta use in agriculture, based particularly on the epidemiological evidence in these reviews and supported by the microbiological evidence.

The current review has been carried out to support the revision of the WHO guidelines in 2003, and the setting of guidelines for wastewater use in Europe by the EU. The aim was to review the adequacy of the available epidemiological information for derivation of guidelines for safe waste reuse in agriculture and to propose new guidelines for waste reuse if the information was adequate. The main health outcomes to be considered were gastro-intestinal infections including intestinal parasite infections (intestinal nematodes and protozoa), specific bacterial infections (e.g. cholera, typhoid), specific viral infections (enteroviruses, hepatitis A) and symptomatic diarrhoeal disease. Epidemiological studies from the existing literature were selected with a view to evaluating the following relationships (i) the effect of consumption of uncooked vegetables irrigated with untreated wastewater on health outcomes (ii) the effect of consumption of uncooked vegetables irrigated with treated wastewater (treatment type specified) on health outcomes (iii) the effect of direct contact with untreated wastewater on health outcomes in farm workers and their families (iv) the effect of direct contact with treated wastewater on health outcomes in farm workers and their families, and (v) the effect on health outcomes in nearby populations of exposure to treated wastewater aerosols.

2. Selection of studies and methods

Information on epidemiological literature pre-1985 was taken from previous intensive literature reviews. Searching of databases (primarily Medline, Cabhealth and Embase plus Healthstar, Popline and Science Citation Index) was used to identify epidemiological studies published between 1985-2000. A wide variety of keywords were used to search on the source of contamination, the type of reuse, the target groups, and health-related terms and health outcomes. This search was supplemented by other studies known to the authors, either published or unpublished. Studies of the effects of wastewater exposure on wastewater treatment plant workers were excluded, as the exposure was (i) not related to agricultural work, and (ii) difficult to categorise (as exposure to untreated and treated wastewater could have occurred).

Due to the relative paucity of epidemiological studies on the health effects of the use of wastewater or excreta in agriculture, all epidemiological studies found were initially included. Studies that met the following criteria were excluded (i) the recall period for the health outcome was over 3 months, (ii) the study relied on the use of hospital data but where the hospital is not the only health facility in the area, (iii) the reported data were not adequate to calculate a relative risk (based on prevalence or incidence comparisons) for the relationship of interest and (iv) preliminary results of studies where the full analysis was reported in another paper. Outbreak investigations are referred to in the text, where the data fill in gaps left by population-based epidemiological studies. Where prevalence data were reported but there was no calculation of a measure of association between exposure and infection, a crude relative risk and 95% confidence
interval was calculated (where the data given allowed) to enable comparisons between studies to be done more easily. Significance levels were reported using p-values at three levels (p<0.05 denoted by *, p<0.01 denoted by **, p<0.001 denoted by *** in the tables).

A sub-set of the most methodologically sound studies was selected using the following criteria:-

(i) study design and implementation included measures to reduce selection and information bias,
(ii) the relationships detected were statistically significant after allowance for the effect of confounding factors or the study design reduced the effect of confounding

For these studies, attributable risks were calculated to estimate the public health importance of the effect reported. This included the attributable risk (that is, the absolute risk of disease in the exposed group that is attributable to exposure), and the population attributable risk percent or population attributable fraction (PAF), showing the proportion of disease in the study population that is attributable to exposure and could be potentially eliminated if exposure were eliminated (sometimes called the preventable fraction).

3. Description and quality of selected studies

In this review, 10 studies of the effect of consumption of uncooked vegetables irrigated with untreated and treated wastewater on health outcomes were selected (Table 1), 25 studies of the effect on health outcomes in farm workers and their families of direct contact with untreated and treated wastewater and the effect on health outcomes in nearby populations of exposure to treated wastewater aerosols (Tables 2 and 3) and 7 studies of the effect of exposure to excreta or nightsoil used as fertilizer (Table 4). Where a study includes more than one type of outcome (e.g. *Ascaris lumbricoides* infection and symptomatic diarrhoeal disease), then it is counted as two studies for the sake of this review.

The studies were categorized according to the type of epidemiological study design, and according to various criteria that would affect the quality of the studies (a) exposure classification (b) disease classification (c) testing for statistical significance (d) control for potential confounding factors and (e) availability of water quality estimates (Tables 1, 2, 3 and 4). Descriptive studies can provide only suggestive data on the relationship between waste reuse and health outcomes, and cannot be used to test specific epidemiological hypotheses. Analytical cross-sectional studies can provide estimates of the associations of interest, but can have limitations since (i) the information on risk factors and outcomes is assessed simultaneously, so causation cannot be established (ii) the current exposure may not reflect the aetiologically relevant exposure (iii) recall of past exposure may not be reliable. So, there can be misclassification of exposure, the direction of which cannot be predicted. However, useful information can be obtained where assessment is of the aetiologically relevant exposure, and the exposure is defined such that recall is more accurate. Prospective cohort studies can assess the effect of exposure on occurrence of infection or disease over a specific time period (e.g. one year). Problems can occur through differences in the composition of exposure groups and through loss-to-follow up.

Of the 10 studies of risks to consumers of vegetable crops irrigated with wastewater (Table 1), there are 5 studies of intestinal helminth infections (mainly *Ascaris* infection)8-13, 2 studies of bacterial infections14,15, 1 study of a virus infection15 and 2 studies of symptomatic diarrhoeal disease12,15. There are 3 descriptive studies8,11, 3 cross-sectional studies12,14,15 (analytical), and 2
Table 1  
Selected studies of risks to consumer of uncooked vegetables irrigated with wastewater

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Study design</th>
<th>Sample size (total)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khalil8</td>
<td>1931</td>
<td>Egypt</td>
<td>Untreated</td>
<td>Ascaris, Trichuris</td>
<td>Descriptive</td>
<td>n/a</td>
<td>a, c,d</td>
</tr>
<tr>
<td>Baumhagger9, Krey10</td>
<td>1949</td>
<td>Germany</td>
<td>Untreated and Treated</td>
<td>Ascaris</td>
<td>Descriptive</td>
<td>n/a</td>
<td>a,c,d,e</td>
</tr>
<tr>
<td>Shuval11</td>
<td>1984</td>
<td>Israel</td>
<td>Untreated</td>
<td>Ascaris</td>
<td>Descriptive</td>
<td>n/a</td>
<td>a,c,d</td>
</tr>
<tr>
<td>Cifuentes12</td>
<td>1998</td>
<td>Mexico</td>
<td>Untreated and treated</td>
<td>Ascaris</td>
<td>Cross-sectional</td>
<td>7294</td>
<td>a</td>
</tr>
<tr>
<td>Peasey13</td>
<td>2000</td>
<td>Mexico</td>
<td>Untreated and Treated</td>
<td>Ascaris</td>
<td>Prospective cohort</td>
<td>980</td>
<td></td>
</tr>
<tr>
<td>Hopkins14</td>
<td>1993</td>
<td>Chile</td>
<td>Untreated</td>
<td>Helicobacter pylori</td>
<td>Cross-sectional</td>
<td>1815</td>
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<tr>
<td>Cifuentes12</td>
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<td>Mexico</td>
<td>Untreated and treated</td>
<td>Diarrhoeal disease</td>
<td>Cross-sectional</td>
<td>7294</td>
<td>a,b</td>
</tr>
<tr>
<td>Blumenthal15</td>
<td>2001a</td>
<td>Mexico</td>
<td>Treated</td>
<td>Diarrhoeal disease</td>
<td>Cross-sectional</td>
<td>4157</td>
<td>b</td>
</tr>
<tr>
<td>Blumenthal15</td>
<td>2001a</td>
<td>Mexico</td>
<td>Treated</td>
<td>Norwalk virus</td>
<td>Prospective cohort</td>
<td>1557</td>
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<td>Blumenthal15</td>
<td>2001a</td>
<td>Mexico</td>
<td>Treated</td>
<td>ETEC</td>
<td>Prospective cohort</td>
<td>1557</td>
<td></td>
</tr>
</tbody>
</table>

a: exposure not defined as consumption of uncooked vegetables at individual level  
b: disease status from self-reported illness or clinic records  
c: significance test not reported in original paper  
d: no control for potential confounding factors  
e: quality of treated wastewater not specified  
n/a not available

prospective cohort studies12,15. The descriptive studies (of Ascaris infection) can provide only suggestive data on the relationship between waste reuse and Ascaris infection. In such studies8-11, the exposure was not defined as the consumption of uncooked vegetables at an individual (or household) level, and there was no estimate of the statistical significance of the exposure-disease relationship, and no control for potential confounding factors. In the analytical cross-sectional studies, different variables were used to measure the consumption of vegetables. One study (of Ascaris infection and diarrhoeal disease)12 was not specifically designed to assess the risk from consumption of vegetables and used proxy measures for consumption of wastewater irrigated vegetables (growing salad in family plot, consumption of local vegetables), a second (on Helicobacter pylori)14 measured only current consumption of vegetables, whereas a third (on diarrhoeal disease)15 assessed frequency of consumption of individual types of vegetable, and assessed both current and ‘usual’ consumption patterns. Prospective cohort studies (of Ascaris infection and Norwalk virus and enterotoxigenic Escherichia coli infection) give better estimates of the relationship between exposure and disease but can suffer from selection bias due to loss-to-follow up which can occur, for example, through failure to give sufficient human samples (stool, blood)13,15. Five studies12-15 reported controlling for risk factors related to the health outcome that could have been potential confounding factors. Confounding factors included age, gender, socio-
economic status (various measures), shellfish consumption, direct contact with wastewater, swimming or bathing in contaminated waters, rubbish disposal and hygiene factors.

Of the 25 studies of risks to agricultural workers and their families, or populations living nearby spray irrigated fields, there were 9 studies of intestinal parasite infections (Table 2), 8 studies of reported enteric infections or symptomatic diarrhoeal disease, and 7 serological studies of (mainly) enteric viral infections (some studies had more than one health outcome) (Table 3). There is one study of Salmonella infection in stool samples. The studies of parasitic infections were mainly cross-sectional (7 studies)\textsuperscript{12,16-18, 20, 21-22} and measured point prevalence of infection; there was one prospective cohort study\textsuperscript{13} which measured prevalence of re-infection after treatment (period prevalence) and intensity of infection. The cross-sectional studies varied in quality: in three studies\textsuperscript{16-18} prevalence data was given for exposed and control groups, but a measure of association was not calculated, there was no significance testing, and there was no control for potential confounding factors. In four cross-sectional studies\textsuperscript{12,20,21,22} (Ascaris and Giardia infection) and 1 prospective cohort study\textsuperscript{13} (Ascaris infection), multivariate analysis was used to calculate a measure of association, with confidence intervals and significance levels, and with allowance for potential confounding factors. The studies of reported enteric infections or symptomatic diarrhoeal disease included two historical cohort studies\textsuperscript{23,24} three prospective cohort studies\textsuperscript{25,26,27}, and three cross-sectional studies\textsuperscript{12,15,20}. The historical cohort studies have limitations due to the difficulty in getting accurate estimates of past exposure, the lack of measurement of potential confounding factors at an individual level, and the reliance on clinic records of variable quality.

The 7 serological studies of viral and bacterial infections (Table 3) include 2 cross-sectional studies (of Legionella infection and poliovirus)\textsuperscript{29,30} and 5 prospective cohort studies (of a range of enteroviruses, rotavirus, Human Norwalk-like Virus / Mexico, and ETEC)\textsuperscript{15,26,28,31,32}. In the prospective cohort studies, paired samples allowed estimates of seroconversion to be made as well as seropositivity. Seroconversion was defined as the number of seronegatives that became seropositives\textsuperscript{29}, a two-fold or greater increase in antibody titre\textsuperscript{32}, and a four-fold or greater increase in antibody titre\textsuperscript{31}; the latter definitions indicated evidence of infection by the agent concerned during the interval between blood collections. In the study of Human Norwalk-like Virus Mexico\textsuperscript{15}, seroresponse was defined as a 50% rise in antibody concentration between paired sera. This was the only study where the estimate of association between exposure and serological outcome was controlled for potential confounding factors. In the other studies, seroconversion could have been due to many other factors besides exposure to wastewater. The sample size in some studies was too low to detect small differences (relative risk, RR <1.5) between serological responses to enteroviruses in different exposure groups at a significant level, especially where seropositivity or seroconversion rates were low\textsuperscript{26,31,32}. Exposure measurement was at the individual level in 5 studies\textsuperscript{15,26,30,31,32}, including the measurement of a sophisticated aerosol exposure index (taking into account microbiological content of wastewater and aerosols) which was calculated for each participant in the Lubbock Infection Surveillance Study\textsuperscript{31}.

There are fewer epidemiological studies of the health effects of use of excreta or ‘nightsoil’ as fertilizer for edible crops (Table 4). Most studies are of geo-helminth infections. Two cross-sectional studies provide data on prevalence for exposed and control groups\textsuperscript{34,36}. A third study assessed the effect of the degree of storage of faeces before use as fertiliser on the intensity of hookworm infection in young women\textsuperscript{35}. In these studies, no measure of association was initially calculated, there was no significance testing, and there was no control for potential confounding factors. Two experimental studies explored the effect of treatment of the excreta with ovicide before use. The positive conversion rate (after chemotherapy) was calculated after a period of exposure. Where baseline prevalence rates were similar in treatment and control groups, positive
<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Treatment and type of irrigation</th>
<th>Outcome</th>
<th>Study design</th>
<th>Sample size</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Krishnamoorti</td>
<td>1973</td>
<td>India</td>
<td>Untreated Flood</td>
<td>Ascaris, hookworm</td>
<td>Cross-sectional</td>
<td>898</td>
<td>a3,c,d</td>
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<td>Bouhoum</td>
<td>1998</td>
<td>Morocco</td>
<td>Untreated</td>
<td>Ascaris, Trichuris</td>
<td>Cross-sectional</td>
<td>528</td>
<td>a1,c,d</td>
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<td>Habbari</td>
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<td>Morocco</td>
<td>Untreated</td>
<td>Ascaris, Trichuris</td>
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<td>a3,c,d</td>
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<td>Srivastava</td>
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<td>Mexico</td>
<td>Treated and untreated Flood/furrow</td>
<td>Ascaris</td>
<td>Prospective cohort</td>
<td>980</td>
<td>a3</td>
</tr>
<tr>
<td>Sehgal</td>
<td>1991</td>
<td>India</td>
<td>Treated and untreated</td>
<td>Giardia</td>
<td>Cross-sectional</td>
<td>2,372</td>
<td>a1,c,d</td>
</tr>
<tr>
<td>Cifuentes</td>
<td>2000</td>
<td>Mexico</td>
<td>Treated and untreated Flood/furrow</td>
<td>Giardia</td>
<td>Cross-sectional</td>
<td>6,748</td>
<td>a3</td>
</tr>
<tr>
<td>Katzenelson</td>
<td>1976</td>
<td>Israel</td>
<td>Treated Sprinkler</td>
<td>Salmonellosis, shigellosis, typhoid fever, infectious hepatitis</td>
<td>Retrospective</td>
<td>82,825</td>
<td>a2,b,c,d,e</td>
</tr>
<tr>
<td>Fattal</td>
<td>1986</td>
<td>Israel</td>
<td>Treated Sprinkler</td>
<td>Enteric disease</td>
<td>Retrospective cohort</td>
<td>3,040</td>
<td>a2,a1,b</td>
</tr>
<tr>
<td>Shuval</td>
<td>1989</td>
<td>Israel</td>
<td>Treated Sprinkler</td>
<td>Enteric disease</td>
<td>Prospective cohort</td>
<td>10,231</td>
<td>a2,a1,b,c</td>
</tr>
<tr>
<td>Linneman</td>
<td>1984</td>
<td>USA</td>
<td>Treated Sprinkler</td>
<td>Clinical illness</td>
<td>Prospective cohort</td>
<td>76</td>
<td>a3,a4,b,d,e</td>
</tr>
<tr>
<td>Camann</td>
<td>1987</td>
<td>USA</td>
<td>Treated Sprinkler</td>
<td>Clinical viral infections</td>
<td>Prospective cohort</td>
<td>233</td>
<td>a4,b</td>
</tr>
<tr>
<td>Blumenthal</td>
<td>2001b</td>
<td>Mexico</td>
<td>Treated and untreated Flood/furrow</td>
<td>Diarrhoeal disease</td>
<td>Cross-sectional</td>
<td>6,256</td>
<td>a3,b</td>
</tr>
<tr>
<td>Cifuentes</td>
<td>1998</td>
<td>Mexico</td>
<td>Treated and untreated Flood/furrow</td>
<td>Diarrhoeal disease</td>
<td>Cross-sectional</td>
<td>7,623</td>
<td>a3,b</td>
</tr>
<tr>
<td>Blumenthal</td>
<td>2001a</td>
<td>Mexico</td>
<td>Treated Flood/furrow</td>
<td>Diarrhoeal disease</td>
<td>Cross-sectional</td>
<td>4157</td>
<td>a3,b</td>
</tr>
</tbody>
</table>

a1: Exposure defined as belonging to group/living in an area with exposure to wastewater (group level)
a2: Exposure defined as living in an area with exposure to wastewater aerosols (group level)
a3: Exposure defined as direct contact with wastewater at an individual level
a4: Exposure defined as exposure to wastewater aerosols at an individual level
a5: Exposure related to protection measures
b: disease status from self-reported illness or clinic records
c: significance test not reported in original paper
d: no control for potential confounding factors
e: quality of treated wastewater not specified
Table 3  Selected serological studies on risks to farm workers and their families exposed to untreated or treated wastewater in agriculture (listed in order of country of study and outcome, viruses before bacteria)

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Treatment and type of irrigation</th>
<th>Outcome</th>
<th>Study design</th>
<th>Sample size</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fattal²⁸</td>
<td>1987</td>
<td>Israel</td>
<td>Treated Sprinkler</td>
<td>Enteric viruses</td>
<td>Prospective cohort</td>
<td>777</td>
<td>a1,a2, c</td>
</tr>
<tr>
<td>Margalith²⁹</td>
<td>1990</td>
<td>Israel</td>
<td>Treated Sprinkler</td>
<td>Poliovirus</td>
<td>Cross-sectional</td>
<td>1,800</td>
<td>a2,c,d,e</td>
</tr>
<tr>
<td>Fattal³⁰</td>
<td>1985</td>
<td>Israel</td>
<td>Treated Sprinkler</td>
<td>Legionella</td>
<td>Cross-sectional</td>
<td>852</td>
<td>a3,d</td>
</tr>
<tr>
<td>Camann³¹</td>
<td>1986</td>
<td>USA</td>
<td>Treated Sprinkler</td>
<td>Enteroviruses</td>
<td>Prospective cohort</td>
<td>478</td>
<td>a4</td>
</tr>
<tr>
<td>Ward³²</td>
<td>1989</td>
<td>USA</td>
<td>Treated Sprinkler</td>
<td>Rotavirus</td>
<td>Prospective cohort</td>
<td>368</td>
<td>a4</td>
</tr>
<tr>
<td>Linnemann²⁹</td>
<td>1984</td>
<td>USA</td>
<td>Treated Sprinkler</td>
<td>Enteroviruses</td>
<td>Prospective cohort</td>
<td>76</td>
<td>a3,a4,d,e</td>
</tr>
<tr>
<td>Blumenthal¹⁵</td>
<td>2001a</td>
<td>Mexico</td>
<td>Treated Flood/furrow</td>
<td>Norwalk-like virus Mexico</td>
<td>Prospective cohort</td>
<td>1557</td>
<td>a3</td>
</tr>
</tbody>
</table>

Table 4  Selected studies of risks to consumer of uncooked vegetables and workers exposed to use of excreta or night soil in agriculture (listed in order of outcome and country of study)

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Study design</th>
<th>Sample size (total)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anders³⁴</td>
<td>1952</td>
<td>Germany</td>
<td>Septic tank contents</td>
<td>Ascaris</td>
<td>Cross-sectional</td>
<td>n/a</td>
<td>a1,c,d,e</td>
</tr>
<tr>
<td>Kreuz citing Harmsen³⁵</td>
<td>1955, 1953</td>
<td>Germany</td>
<td>Untreated</td>
<td>Ascaris</td>
<td>Cross-sectional</td>
<td>144 families</td>
<td>a2</td>
</tr>
<tr>
<td>Kozai³⁷</td>
<td>1962</td>
<td>Japan</td>
<td>Treatment with ovicide</td>
<td>Ascaris</td>
<td>Experimental</td>
<td>608</td>
<td>a2,c,d,e</td>
</tr>
<tr>
<td>Kutsumi³⁸</td>
<td>1969</td>
<td>Japan</td>
<td>Treatment with ovicide</td>
<td>Ascaris and Trichuris</td>
<td>Experimental</td>
<td>732, 920</td>
<td>a2,c,d,e</td>
</tr>
<tr>
<td>Kozai³⁷</td>
<td>1962</td>
<td>Japan</td>
<td>Treatment with ovicide</td>
<td>Hookworm</td>
<td>Experimental</td>
<td>299</td>
<td>a2,c,d,e</td>
</tr>
<tr>
<td>Kutsumi³⁸</td>
<td>1969</td>
<td>Japan</td>
<td>Treatment with ovicide</td>
<td>Hookworm</td>
<td>Experimental</td>
<td>1384</td>
<td>a2,c,d,e</td>
</tr>
<tr>
<td>Humphries³⁹</td>
<td>1997</td>
<td>Vietnam</td>
<td>Treatment with ash and storage</td>
<td>Hookworm</td>
<td>Cross-sectional</td>
<td>217</td>
<td>a3</td>
</tr>
</tbody>
</table>

a1: Exposure defined as belonging to group/living in an area with exposure to wastewater (group level)
a2: Exposure defined as living in an area with exposure to wastewater aerosols (group level)
a3: Exposure defined as direct contact with wastewater at an individual level
a4: Exposure defined as exposure to wastewater aerosols at an individual level
b: disease status from self-reported illness or clinic records
c: significance test not reported in original paper
d: no control for potential confounding factors
e: quality of treated wastewater not specified
n/a: not available
conversion rates were compared in treatment and control groups; where baseline rates were not comparable, rates in the treatment group before and after treatment were compared.

4. **Summary of results**

4.1 **Risks to consumers from wastewater irrigation of crops eaten uncooked**

4.1.1 *Ascaris infection.* Descriptive studies of the association between consumption of uncooked vegetables irrigated with untreated wastewater and *Ascaris* infection produced estimates of relative risks between 7.0 and 35.0 (Table 5). An analytical cross-sectional study provided an estimate of relative risk of 1.4, but it is not clear how much this is a measure of the risk of the use of untreated or treated wastewater, as stratum specific estimates were not reported. A prospective cohort study, however, produced adjusted odds ratios (OR) of 3.9 (men) and 2.4 (children) for consumption of vegetables irrigated with wastewater by farming families, when allowance was made for potential confounding factors for *Ascaris* such as age, gender, socio-economic status, and direct wastewater contact. There is some evidence in adult men that consumption of vegetables irrigated with wastewater had a greater effect when irrigation was with untreated wastewater (OR=2.7, p=0.074) as compared to treated wastewater (OR=0.6, p=0.68) was used, but this did not reach statistical significance. In children, irrigation of vegetables with untreated wastewater was associated with an increased risk of infection (OR=4.2, p=0.001) and that use of treated wastewater (<1 nematode eggs/litre) was also associated with an increased risk of infection (OR=3.7, p=0.056), but the effect was of borderline significance. The latter may have been due to the small number of individuals who did not eat vegetables, and the low number of positive cases (2/15). A descriptive (ecological) study provides suggestive evidence that treatment using sedimentation and biological oxidation reduces the risks of *Ascaris* among consumers of uncooked vegetables to below the levels seen where no wastewater irrigation takes place.

4.1.2 *Bacterial and viral infections.* There are fewer studies of the risk of specific bacterial or viral infections associated with consumption of vegetable crops irrigated with untreated wastewater. Consumption of raw vegetables coming from an area where untreated wastewater is used for irrigation (Santiago) was related to an increase in seroprevalence to *Helicobacter pylori* (RR=3.3, p=0.001), when allowance was made for age, gender, socio-economic status, shellfish consumption, swimming near contaminated beaches and bathing in fresh water rivers or irrigation ditches. There is evidence of the transmission of cholera, typhoid, and shigellosis, when vegetables are irrigated with untreated wastewater, from outbreaks of infection in Israel and Chile. Evidence of risks associated with earlier outbreaks of infection (before 1977) have been summarised by Bryan.

There is some evidence that when wastewater is partially-treated (through storage in reservoirs) the risks to consumers of vegetable crops are reduced, but not eliminated. A prospective cohort study in Mexico showed that there was a two-fold increase in seroreponse to Human Norwalk-like Virus / Mexico (Mexico virus) associated with the consumption of green tomatoes irrigated with river water consisting of wastewater that had been stored in a single reservoir (quality 10^4 FC/100ml), when allowance was made for age, gender, socio-economic status, consumption of other vegetables, direct contact with wastewater, and other risk factors for Mexico virus (Table 5). There was no increased risk of Mexico virus associated with consumption of other vegetables. In addition, there was no increased risk of seroresponse to enterotoxigenic *Escherichia coli* infection associated with vegetable consumption. Cross-sectional studies of symptomatic diarrhoeal disease in the same study group indicated that there was a two-fold or greater risk of diarrhoeal disease associated with medium or high frequencies of consumption of uncooked
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome and outcome measure</th>
<th>Wastewater quality</th>
<th>Age group (years)</th>
<th>Prevalence of outcome measure (%)</th>
<th>Relative risk or odds ratio (95% CI)</th>
<th>Study group and comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khalil¹ (1931)²</td>
<td>Ascaris (prevalence)</td>
<td>Untreated</td>
<td>Adults 70 vs 10</td>
<td>7.0²</td>
<td></td>
<td>Prison population eat vegetables irrigated with w/w vs village population not using w/w or night soil in agriculture</td>
</tr>
<tr>
<td>Baumogger⁹ (1949), Krey¹⁰ (1949)²</td>
<td>Ascaris (prevalence – routine data)</td>
<td>Untreated</td>
<td>All ages 50 vs 6</td>
<td>8.3²</td>
<td></td>
<td>City where untreated w/w used to irrigate vegetables vs average of 5 cities with no w/w irrigation</td>
</tr>
<tr>
<td>Cifuentes¹² (1998)</td>
<td>Ascaris (prevalence)</td>
<td>Untreated and treated (storage reservoirs)</td>
<td>5+</td>
<td>4.9 vs 2.9</td>
<td>1.43 (1.07-1.92)²</td>
<td>Study population. Consumption of local vegetables (w/w irrigated fields) vs vegetables grown outside village</td>
</tr>
<tr>
<td>Peasey¹³ (2000)</td>
<td>Ascaris (prevalence)</td>
<td>(i) Untreated and treated (ii) Untreated (iii) Treated &lt; 1 nematode egg &gt; 15 male</td>
<td>&lt; 15</td>
<td>(i) 41.1 vs 16.4 (ii) 46.5 vs 17.3 (iii) 36.5 vs 13.3 (i) 22.4 vs 8.8 (ii) 22.4 vs 9.5 (iii) 8.0 vs 12.5</td>
<td>2.41 (1.07-5.44)² 4.15 (1.89-9.41)*** 3.74 (0.80–17.38) 3.93 (1.01-15.24) 2.74 (0.84-8.81) 0.61 (0.06-5.81)</td>
<td>Study population. Consumption of uncooked vegetables from local (wastewater-irrigated) field or garden Yes vs No (i) adjusted OR’s (ii) and (iii) crude OR’s</td>
</tr>
<tr>
<td>Baumogger⁹ (1949), Krey¹⁰ (1949)²</td>
<td>Ascaris (prevalence)</td>
<td>Treated (sedimentation and biological oxidation)</td>
<td>All ages</td>
<td>2.2 vs 6.1</td>
<td>0.36⁰</td>
<td>City where treated w/w used to irrigate vegetables vs average of 5 cities with no w/w irrigation</td>
</tr>
<tr>
<td>Hopkins¹⁴ (1993)</td>
<td>Helicobacter pylori (seroprevalence)</td>
<td>Untreated</td>
<td>&lt;35</td>
<td>38 vs 9</td>
<td>3.25 (1.94-5.71)***</td>
<td>Study population. Consumption of uncooked vs cooked vegetables</td>
</tr>
<tr>
<td>Cifuentes¹² (1998)</td>
<td>Diarrhoeal disease (self-reported 2-weekly prevalence)</td>
<td>Untreated and treated</td>
<td>5+</td>
<td>19.0 vs 10.0</td>
<td>2.00 (1.37-2.93)***</td>
<td>Study population. Family grows salad (w/w irrigated) vs family not grow salad</td>
</tr>
<tr>
<td>Blumenthal¹⁵ (2001a)</td>
<td>Diarrhoeal disease (self-reported weekly prevalence)</td>
<td>Treated (storage reservoirs) 10³ FC/100ml</td>
<td>1-4</td>
<td>&lt;1/wk 2.6 l/wk 9.5 &gt;1/wk 5.8</td>
<td>1.00 3.80 (1.24-11.68) 2.19 (0.54-8.89) p=0.05</td>
<td>Study population. Medium and high frequencies of consumption of uncooked onions vs low frequency</td>
</tr>
<tr>
<td>Blumenthal¹⁵ (2001a)</td>
<td>Human Norwalk-like virus/Mexico (seroresponse)</td>
<td>Treated (storage reservoirs) 10³ FC/100ml</td>
<td>5-14</td>
<td>0/2wks 13.6 1/2wks 20.6 2-14/2wks 29.6</td>
<td>1.00 1.44 (0.76-2.75) 2.52 (1.03-6.13)</td>
<td>Study population. Different frequencies of consumption of uncooked green tomatoes.</td>
</tr>
</tbody>
</table>

a described in Shuval et al, 1986
b crude relative risk calculated from prevalence data reported
c crude odds ratios and 95% confidence intervals calculated for prevalence data reported
onions irrigated with water from the same river when compared with a low frequency of consumption. However, there was no significant risk related to the total consumption of uncooked vegetables, as eating some vegetables was a protective factor against diarrhoea. In a separate cross-sectional study\textsuperscript{12}, there was a two-fold increased risk of diarrhoeal disease associated with the family growing salad compared to those not growing salad, but it is not clear how much this is a measure of the risk of the use of untreated or treated wastewater, as stratum specific estimates were not reported.

4.1.3 Overall. There is evidence to suggest that the use of untreated wastewater to irrigate vegetables can lead to increased helminth infection (mainly Ascaris lumbricoides infection), bacterial infections (typhoid, cholera, Helicobacter pylori) and symptomatic diarrhoeal disease in consumers. The studies of Ascaris infection among consumers indicate that treatment is needed to reduce the risk of Ascaris infection to consumers of crops irrigated with untreated wastewater. It is not possible to determine the extent of treatment that is needed from the available data, but there is an indication that treatment to ≤1 nematode egg per litre may not be sufficient in certain circumstances, especially where children are exposed. When wastewater is partially-treated, there is evidence that the risk of enteric infections (bacterial and viral origin) is still significant when consumers eat some types of uncooked vegetables irrigated by water exceeding WHO guideline of 1000 FC/100ml by a factor of ten.

4.2 Risks to agricultural workers and their families from direct contact with wastewater and to populations living nearby fields sprinkler-irrigated with wastewater

4.2.1 Intestinal parasitic infections. Results of studies of the effect of flood or furrow irrigation with wastewater on intestinal parasite infections are given in Table 6. Estimates of the effect of direct contact with untreated wastewater compared with no contact with untreated wastewater on Ascaris infection vary from relative risks of 1.5\textsuperscript{13} to 18.0\textsuperscript{20} in children and relative risks of 3.5\textsuperscript{16} to 5.4\textsuperscript{13} in adults (increasing to 13.5 where older children and adults are combined\textsuperscript{20}). The large variation in the estimates for the effect on children is related to the level of infection in the comparison group: high estimates were produced where an external comparison group was used (i.e. children from farming families in a nearby area not using wastewater for irrigation) rather than an internal comparison group (i.e. children from farming families in the same area but who did not have direct contact with wastewater). There may be uncontrolled confounding where an external comparison group has been used. Seasonality in transmission may also affect the estimates; in studies in Mexico, the relative risk estimates were higher in the dry season\textsuperscript{20} than in the rainy season\textsuperscript{12}.

There is some evidence that Ascaris infection can be reduced when wastewater is partially treated before use, but the effect depends on the extent of treatment (Table 6). In studies in Mexico, wastewater retention in one reservoir for a minimum of one month during the year preceding the study (achieving 2 log faecal coliform removal and 2 log nematode egg removal, to <1 nematode egg per litre) was adequate to reduce the risks of Ascaris infection in adults\textsuperscript{13} but not in children\textsuperscript{13,20}. The high relative risk associated with exposure to one reservoir in ‘adults’ in one study\textsuperscript{20} was due to the combination of data from adults and school-aged children – the main effect was actually in those aged 5-14 years. However, retention in two reservoirs in series for a period of one or two months in each reservoir (achieving a 4 log faecal coliform removal and 2-3 log nematode egg removal) resulted in no excess risk of Ascaris in children\textsuperscript{12}. The significant excess risk in adults is of little public health importance, since the attributable risk is 0.8%.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome</th>
<th>Wastewater quality</th>
<th>Age group (years)</th>
<th>Prevalence of infection (%)</th>
<th>Relative risk or odds ratio (95% CI)</th>
<th>Study group and comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krishnamoorti &amp; Bouhouni (1973)</td>
<td>(i) Ascaris, (ii) Hookworm, (iii) Trichuris</td>
<td>Untreated</td>
<td>adults</td>
<td>(i) 46.8 vs 13.4 (ii) 69.7 vs 32.6</td>
<td>3.48 (2.69-4.51)** (2.14 (1.84-2.48))***b</td>
<td>Sewage farm workers vs farm workers exposed to clean water</td>
</tr>
<tr>
<td>Habbari (2000)</td>
<td>Ascaris</td>
<td>Untreated</td>
<td>School children</td>
<td>(i) 33 vs 2 (ii) 17 vs 2</td>
<td>16.3 (8.5)***</td>
<td>School children in urban area where sewage use in irrigation vs where sewage not used</td>
</tr>
<tr>
<td>Srivastava (1986)</td>
<td>(i) Parasitic infection, (ii) Hookworm</td>
<td>Untreated, plus health protection measures</td>
<td>Adult</td>
<td>(i) 81.6 vs 26.2 (ii) 25.1 vs 7.7</td>
<td>3.1 (3.3)***</td>
<td>(i) Sewage farm workers with poor personal hygiene vs good hygiene (ii) Sewage farm workers barefoot vs wearing shoes</td>
</tr>
<tr>
<td>Blumenthal (2001b)</td>
<td>Ascaris</td>
<td>(i) Untreated (ii) Treated (one storage reservoir)</td>
<td>0-4</td>
<td>(i) 10.0 vs 0.6 (ii) 11.8 vs 0.6</td>
<td>18.0 (4.10-79.16)*** (21.22 (5.06-88.93))***</td>
<td>Farm workers or their children (i) in direct contact with raw w/w vs not using w/w for irrigation (dry season) (ii) in direct contact with w/w stored in one reservoir vs not using w/w for irrigation</td>
</tr>
<tr>
<td>Cifuentes (1998)</td>
<td>Ascaris</td>
<td>(i) Untreated (ii) Treated (two storage reservoirs)</td>
<td>0-4</td>
<td>(i) 13.7 vs 2.5 (ii) 3.3 vs 2.5</td>
<td>5.71 (2.44-13.36)*** 1.29 (0.49-3.39)</td>
<td>Farm workers or their children (i) in direct contact with raw w/w vs not using w/w for irrigation (rainy season) (ii) in direct contact with w/w stored in two reservoirs vs not using w/w for irrigation</td>
</tr>
<tr>
<td>Peasey (2000)</td>
<td>Ascaris</td>
<td>(i) Untreated (ii) Treated (one storage reservoir)</td>
<td>&lt;15</td>
<td>(i) 50.0 vs 30.0 (ii) 44.1 vs 30.0</td>
<td>1.50 (0.59-3.79) 2.61 (1.10-6.15)**</td>
<td>Farm workers or their children (i) in direct contact with raw w/w vs not in direct contact with w/w (dry season) (ii) in direct contact with w/w stored in one reservoir vs not in direct contact with w/w [Direct contact was through (a) play (&lt; 15 yrs) (b) work relating to chilli production (ma le &gt; 15 yrs) (c) tending livestock in w/w irrigated fields (female &gt;15 yrs)]</td>
</tr>
<tr>
<td>Sehgal (1991)</td>
<td>Giardia</td>
<td>(i) Untreated (ii) Treated</td>
<td>Not specified</td>
<td>(i) 12.3 vs 11.5 (ii) 15.5 vs 11.5</td>
<td>1.07 (0.85-1.35) 1.35 (0.99-1.86)***</td>
<td>(i) General population in villages using raw w/w for irrigation vs general population in control village not using sewage (ii) General population in villages using treated w/w for irrigation vs general population in control village not using sewage</td>
</tr>
<tr>
<td>Cifuentes (2000)</td>
<td>Giardia</td>
<td>(i) Untreated (ii) Treated</td>
<td>All ages</td>
<td>(i) 8.1 vs 7.8 (ii) 10.9 vs 7.8</td>
<td>1.01 (0.84-1.36) 1.22 (0.94-1.58)</td>
<td>Farm workers and their families (i) in direct contact with raw w/w vs not using w/w for irrigation (ii) in direct contact with w/w stored in two reservoirs vs not using w/w for irrigation</td>
</tr>
</tbody>
</table>

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a described in Shuval et al, 1986
b relative risk and 95% confidence interval calculated from prevalence or incidence rates and population data reported
c crude relative risk calculated from prevalence or incidence data reported
w/w
There is some evidence that *Ascaris* infection can be reduced when wastewater is partially treated before use, but the effect depends on the extent of treatment (Table 6). In studies in Mexico, wastewater retention in one reservoir for a minimum of one month during the year preceding the study (achieving 2 log faecal coliform removal and 2 log nematode egg removal, to <1 nematode egg per litre) was adequate to reduce risks of *Ascaris* infection in adults\textsuperscript{13} but not in children.\textsuperscript{13,20}

The high relative risk associated with exposure to one reservoir in ‘adults’ in one study\textsuperscript{20} was due to the combination of data from adults and school-aged children – the main effect was actually in those aged 5-14 years. However, retention in two reservoirs in series for a period of one or two months in each reservoir (achieving a 4 log faecal coliform removal and 2-3 log nematode egg removal) resulted in no excess risk of *Ascaris* in children\textsuperscript{12}. The significant excess risk in adults is of little public health importance, since the attributable risk is 0.8%.

There are very few data on the effect of contact with wastewater in agriculture on protozoan infections. Studies in India\textsuperscript{21} and Mexico\textsuperscript{22} have produced similar results; that is, that there is no significant risk of *Giardia intestinalis* infection related to contact with untreated or treated wastewater for irrigation. However, in both studies, the effect of contact with treated wastewater is higher than for untreated wastewater; there is no obvious explanation for this.

### 4.2.2 Reported enteric infections

Table 7 shows the results of studies of the effects of wastewater exposure on enteric infections reported to health clinics\textsuperscript{23-25}, and on clinical illness or symptomatic diarrhoeal disease reported to interviewers in field studies\textsuperscript{12,15,20,26,27}. Several studies in Israel have investigated the effect of exposure of the general population to wastewater aerosols from sprinkler irrigation of partially-treated wastewater from waste stabilisation ponds (short retention times as well as the effect of occupational contact with the wastewater. The first study\textsuperscript{23} suggested increases in salmonellosis, shigellosis, typhoid fever and infectious hepatitis in the kibbutz population living near sprinkler irrigated field (water quality 10\textsuperscript{6-8} TC/100ml). Severe methodological problems (described by Fattal\textsuperscript{44}), however, cast doubt on the accuracy of these findings. The second study\textsuperscript{24} found a two-fold excess risk of clinical ‘enteric’ disease in young children living within 600-1000m of sprinkler irrigated fields, when the water quality was very poor (10\textsuperscript{6-8} TC/100ml), but this was in the summer irrigation season only, with no excess illness found on a year round basis. The third study, a prospective cohort study\textsuperscript{25}, found that episodes of enteric disease were similar in kibbutzim most exposed to treated wastewater aerosols when the water quality was better (10\textsuperscript{4-5} TC/100ml; sprinkler irrigation within 300-600m of residential areas) and those not exposed to wastewater in any form. The absence of an effect of exposure in the third study was due to the better quality of the water and the superior epidemiological design (Shuval et al, 1986).

In USA, the Lubbock Infection Surveillance Study (LISS)\textsuperscript{27} measured the frequency of clinical viral infections (through reported illness supplemented by detection of viruses in stool samples) in a population before and after exposure to sprinkler irrigation with treated wastewater. When low quality wastewater (trickling filter plant effluent) was used for irrigation, there was an association between high aerosol exposure and viral illness, but this was of borderline significance (p=0.06, Fisher’s exact test). When allowance was made for potential confounding factors, eating at local restaurants was identified as the risk factor for the viral illness episodes, but there was no significant effect on viral illness of exposure to higher quality wastewater from storage reservoirs (10\textsuperscript{4-5} FC/100ml)\textsuperscript{45}. A similar result was found in another study in USA\textsuperscript{26} using effluent from storage lagoons (where storage was for around 6 months).

In Mexico, flood or furrow irrigation is used. Studies of the effect of direct contact with untreated wastewater (through irrigation, shepherding animals or play)\textsuperscript{12,20} indicate that there was an increased risk of diarrhoeal disease, particularly in young children, and the effect was stronger in
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome and outcome measure</th>
<th>Wastewater quality</th>
<th>Age group (years)</th>
<th>Prevalence or incidence of infection (%)</th>
<th>Relative risk or odds ratio (95% CI)</th>
<th>Study group and comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katzenelson23 (1976)</td>
<td>Ear, nose, throat symptoms</td>
<td>Treated</td>
<td>All ages</td>
<td>(i) 23.4 vs 6.3 (ii) 100.2 vs 45.5 (iii) 1.16 vs 0.27 (iv) 8.8 vs 4.4</td>
<td>3.7 2.2 4.3 2.0</td>
<td>Population in kibbutzim using w/w for irrigation vs not using w/w</td>
</tr>
<tr>
<td>Fattal24 (1986)</td>
<td>Enteric disease (Incidence per 100 person-years)</td>
<td>Treated</td>
<td>(i) 0-4 (ii) 5-18 (iii) ≥19</td>
<td>(i) 51.8 vs 27.4 (ii) 11.2 vs 6.6 (iii) 4.7 vs 1.8</td>
<td>1.91 (1.30-2.80) 1.23 (0.46-3.25) 2.06 (0.69-6.16)</td>
<td>Comparison of enteric disease rates in kibbutzim when using w/w for sprinkler irrigation vs when not using w/w for irrigation, with allowance made for rate of control diseases and other factors. Results from irrigation season.</td>
</tr>
<tr>
<td>Shuval25 (1989)</td>
<td>Enteric disease (Incidence per 100 person-years)</td>
<td>Treated</td>
<td>(i) All ages (ii) 0-5</td>
<td>L 11.0 M 9.4 H 11.6 L 26.4 M 20.0 H 26.0</td>
<td>1.00 0.85 1.05 1.00 0.76 0.98</td>
<td>Comparison of rates in kibbutzim population with w/w sprinkler irrigation within 300-600m (High=H) or kibbutzim with w/w use but no aerosols (Medium=M) vs kibbutzim with no use of w/w (Low=L). [No excess enteric disease was seen in wastewater contact workers or their families as compared with the unexposed]</td>
</tr>
<tr>
<td>Linnemann26 (1984)</td>
<td>Clinical illness (Incidence per worker month)</td>
<td>Treated. Storage lagoons (over winter)</td>
<td>Adults</td>
<td>0.54 vs 0.58</td>
<td>0.93</td>
<td>Comparison of illness rates in spray irrigation workers vs road commission workers (no significant differences found with level of exposure).</td>
</tr>
<tr>
<td>Camann27 (1987)</td>
<td>Clinical viral infections (% with infection episode in irrigation season)</td>
<td>Treated (summer 1982- trickling filter plant effluent 8x10^6 FC/100ml, 3.2 EV/100ml; summer 1983- effluent from TFP and storage reservoirs 10^3 FC/100ml, 0.4 EV/100ml)</td>
<td>Adults and children &lt;13</td>
<td>Summer 1982 (i) 8 (ii) 8 (iii) 24</td>
<td>p=0.06</td>
<td>Comparison of faecal donors with, (i) low, (ii) medium, and (iii) high aerosol exposure</td>
</tr>
<tr>
<td>Blumenthal15 (2001a)</td>
<td>Diarrhoal disease (weekly prevalence)</td>
<td>Treated (two reservoirs)</td>
<td>0-4</td>
<td>(i) 19.4 vs 13.6 (ii) 15.6 vs 13.6 (iii) 7.1 vs 5.9</td>
<td>1.75 (1.10-2.78)** 1.13 (0.70-1.83) 1.34 (1.00-1.78)* 1.50 (1.15-1.96)**</td>
<td>Farm workers or their children (i) in direct contact with raw w/w vs not using w/w for irrigation (dry season) (ii) in direct contact with w/w in one reservoir vs not using w/w for irrigation</td>
</tr>
<tr>
<td>Cifuentes12 (1998)</td>
<td>Diarrhoeal disease (2-weekly prevalence)</td>
<td>(i) Untreated and (ii) Treated (two storage reservoirs) 10^3 FC/100ml</td>
<td>0-4</td>
<td>(i) 29.0 vs 23.0 (ii) 26.8 vs 23.0 (iii) 11.8 vs 9.8 (iv) 10.5 vs 9.8</td>
<td>1.33 (0.96-1.85) 1.17 (0.85-1.60) 1.10 (0.88-1.38) 1.06 (0.86-1.29)</td>
<td>Farm workers or their children (i) in direct contact with raw w/w vs not using w/w for irrigation (rainy season) (ii) in direct contact with w/w in two reservoirs vs not using w/w for irrigation</td>
</tr>
<tr>
<td>Blumenthal13 (2001a)</td>
<td>Diarrhoeal disease (weekly prevalence)</td>
<td>Treated (two reservoirs) 10^3 FC/100ml</td>
<td>5-14</td>
<td>(i) 11.0 vs 4.0 (ii) 7.4 vs 3.2</td>
<td>3.05 (1.67-5.58)** 2.34 (1.20-4.57)*</td>
<td>Children of farm workers with, (i) high level of contact with w/w vs no contact (rainy season) (ii) contact with w/w vs no contact (dry season)</td>
</tr>
</tbody>
</table>

a crude relative risk calculated from prevalence or incidence data reported
the dry season\textsuperscript{20} than the rainy season\textsuperscript{12}. When wastewater was partially-treated in one reservoir\textsuperscript{20}, there was still an excess risk of diarrhoeal disease in the dry season, but when it was treated in two reservoirs in series\textsuperscript{12}, no excess risk was detected in the rainy season. In a later study in the same (two reservoirs) area\textsuperscript{15}, however, an increased risk of diarrhoeal disease was found in school-aged children in the dry season, and in children with a high level of contact in the rainy season when compared with children with no contact with wastewater. This may be due to seasonal effects, using an internal not external comparison group, or using an individual measure of exposure for children\textsuperscript{15} instead of assigning children the father’s exposure\textsuperscript{12,20}.

4.2.3 \textit{Serological studies of viral and bacterial infections.} Data from serological studies of the effect of wastewater exposure on viral and bacterial infections are given in Table 8. Serological studies from kibbutzim in Israel\textsuperscript{28-30} indicate that there is no excess endemic viral infection related to exposure to wastewater aerosols through sprinkler irrigation from 5-10 day waste stabilization ponds (wastewater quality 10\textsuperscript{6-8} TC/100ml). There was no significant excess of antibodies to echovirus types 7 and 9, coxsackie virus types A9, B1, B3 and B4 and hepatitis A virus\textsuperscript{28}, or poliovirus types 1, 2 and 3\textsuperscript{29}. However, there was a significant excess of antibodies to echovirus type 4 (but no excess disease), in kibbutzim that had been exposed to aerosols of partially treated wastewater from nearby towns\textsuperscript{28}. This was attributed to there being a major national epidemic of echovirus 4, which peaked shortly before the collection of blood samples. This indicates that viral infection can be spread through wastewater use when there are high concentrations of viruses in the wastewater, as in an epidemic, but this may not cause disease where the infection is endemic. In a related study\textsuperscript{30}, there was no significant excess seropositivity to \textit{Legionella} organisms in sewage contact workers compared with non-sewage irrigation or fish pond workers, or with control group workers (though the sample size was too small to detect significant effects).

In the Lubbock Infection Surveillance Study (LISS)\textsuperscript{31} in USA, blood samples were analysed for 15 enteroviruses (coxsackie viruses, echoviruses and polioviruses) and influenza A (control virus). Wastewater irrigation was significantly associated with new viral infections, when seroconversion incidence densities for coxsackie B and echo viruses (recovered concurrently from wastewater) were compared before and after irrigation started (Table 8)\textsuperscript{46}. The association was stronger and more highly significant in those who had a high degree of aerosol exposure following irrigation. In the period following the start of irrigation, excess viral infection was associated with high wastewater aerosol exposure (as measured through an individual cumulative exposure index) based on the comparison of crude seroconversion incidence densities by aerosol exposure level. The effect only reached statistical significance when high and intermediate exposure were compared. However, there is no published estimate of a relative risk of disease related to exposure adjusted for potential confounding factors. Further analysis was done of particular infection episodes, that is, when a number of infections by the same agent were observed in the study population within a restricted time interval, such as, an irrigation period. Infection episodes (n=5) that were significantly associated with aerosol exposure occurred mainly in 1982, when effluent from a trickling filter plant (quality 10\textsuperscript{6} FC/100ml, 100-1000 enterovirus/100ml) was being used for irrigation, and not in 1983 (n=1), when effluent from storage reservoirs (quality 10\textsuperscript{-4} FC/100ml, <10 enterovirus/100ml) were used. However, when allowance was made for potential confounding factors (such as eating in local restaurants, farmer as head of household, low income, large household) the association between exposure and infection was only significant (at p<0.05 level) for 2 out of the 5 episodes reported\textsuperscript{46} where the agents of infection were poliovirus 1, and coxsackieB and echoviruses (recovered concurrently from wastewater). In a study of rotavirus infection\textsuperscript{12} (within LISS study) there was no significant difference in seroconversion when irrigation was with effluent from storage reservoirs as compared to when it was effluent from a trickling filter plant. However, the percentage of seroconversion for rotavirus was actually significantly less after irrigation with effluent was started than before it started (RR=0.37,
Table 8  Studies of risks to workers and nearby populations – enteric viruses and bacteria - seroprevalence and seroconversion in populations exposed vs non-exposed to wastewater for irrigation and relative risks

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome (seroprevalence and seroconversion)</th>
<th>Wastewater quality</th>
<th>Age group (years)</th>
<th>Seroprevalence (%) or seroconversion (%)</th>
<th>Relative risk or odds ratio (95% CI)</th>
<th>Study group and comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fattal 26 (1987)</td>
<td>Echovirus type 4 (i) Polio 1 (ii) Polio 2 (iii) Polio 3</td>
<td>Treated Stabilization ponds 5-10 day detention 10&lt;sup&gt;6&lt;/sup&gt; TC/100ml</td>
<td>(i) 0-5 (ii) 6-17 (iii) 25+</td>
<td>Seroprevalence (i) 83 vs 33 (ii) 73 vs 37 Seroconversion (iii) 63 vs 20</td>
<td>2.5*** 2.0** 3.2**</td>
<td>Comparison of rates in kibbutzim population exposed to aerosolized w/w from kibbutz itself and nearby towns vs kibbutzim not exposed to w/w (other comparisons given in paper) Data from 1980 presented – after national outbreak of echovirus 4 No significant differences were found for all other enteroviruses</td>
</tr>
<tr>
<td>Margalith 29 (1990)</td>
<td>Poliovirus (i) 1 (ii) 2 (iii) 3</td>
<td>Treated Stabilization ponds 5-10 day detention</td>
<td>&lt;1 - 60+</td>
<td>(i) 82 vs 86 (ii) 88 vs 91 (iii) 80 vs 82</td>
<td>0.95 a 0.97 0.98</td>
<td>1980 data shown Comparison of rates in kibbutzim population exposed to aerosolized w/w from kibbutz itself and nearby towns vs kibbutzim not exposed to w/w</td>
</tr>
<tr>
<td>Fattal 30 (1985)</td>
<td>Legionella pneumophila</td>
<td>Treated Stabilization ponds 5-7 day detention 10&lt;sup&gt;4&lt;/sup&gt; TC/100ml</td>
<td>18+</td>
<td>4.3 vs 1.4</td>
<td>3.14 (0.89-11.85) a</td>
<td>Sewage contact workers vs non-irrigation workers</td>
</tr>
<tr>
<td>Camann 31 (1986)</td>
<td>Enteric viruses (Seroconversion incidence densities)</td>
<td>Treated Trickling filter effluent 10&lt;sup&gt;6&lt;/sup&gt; FC/100ml (1982) Reservoir effluent 10&lt;sup&gt;1&lt;/sup&gt;-10&lt;sup&gt;6&lt;/sup&gt; FC/100ml (1983)</td>
<td>All ages</td>
<td>(i) 5.37 vs 2.55 (ii) 8.34 vs 1.32 (iii) 8.34 vs 5.46 (iv) 8.34 vs 4.68</td>
<td>2.10 (1.56-2.03) b 6.31 (2.52-15.76)*** 1.56 (0.86-2.84) 1.78 (1.03-3.09)*</td>
<td>(i) Irrigation vs baseline (ii) High exposure: irrigation vs baseline (iii) Irrigation: High aerosol exposure (index&gt;5) vs low (index&lt;1) (iv) Irrigation: High aerosol exposure vs intermediate aerosol exposure (1≤ index ≤ 5)</td>
</tr>
<tr>
<td>Ward 32 (1989)</td>
<td>Rotavirus (% seroconversion )</td>
<td>Treated – trickling filter plant then storage reservoirs</td>
<td>All ages</td>
<td>(i) 1.99 vs 5.34 (ii) 1.54 v 2.50</td>
<td>0.37 (0.23-0.62)**** 1.63 (0.70-3.78) b</td>
<td>(i) 20 months post- start of spray irrigation vs 20 months before irrigation (ii) 10 months spray irrigation with reservoir effluent vs 10 months spray irrigation with trickling filter plant effluent</td>
</tr>
<tr>
<td>Linnemann 33 (1984)</td>
<td>Enteroviruses – coxsackievirus B5 (% seroprevalence)</td>
<td>Treated. Storage lagoons (over autumn and winter)</td>
<td>Adults</td>
<td>100 vs 52</td>
<td>1.93 (1.36-2.75)*</td>
<td>Comparison of seropositivity in spray irrigation workers vs spray nozzle cleaners No significant differences were found for all other enteroviruses</td>
</tr>
<tr>
<td>Blumenthal 34 (2001a)</td>
<td>Norwalk-like virus Mexico (% seroresponse)</td>
<td>Treated 10&lt;sup&gt;4&lt;/sup&gt; FC/100ml</td>
<td>&gt;15</td>
<td>33.3 vs 11.4</td>
<td>4.21 (1.62-10.96)**</td>
<td>Farm workers with high level of contact with w/w vs no contact</td>
</tr>
<tr>
<td>Ait Melloul 35 (1999)</td>
<td>Salmonella infection (prevalence)</td>
<td>Untreated</td>
<td>&lt; 15</td>
<td>39.3 vs 24.6</td>
<td>1.60***</td>
<td>Children of agriculturalists vs children from non-agricultural families</td>
</tr>
</tbody>
</table>

a  crude relative risk calculated from seroprevalence or seroconversion data reported
b  crude relative risk and 95% confidence interval calculated from seroprevalence or seroconversion
p<0.001) suggesting that the results were affected by residual confounding and are not credible. In an earlier study, no significant differences in seroresponse to infections were found in spray irrigation workers exposed to effluent from storage lagoons except for those who cleaned the nozzles (and were frequently soaked with wastewater), who had higher seroprevalence of Coxsackie B5 virus. In a study in Mexico, however, farm workers who were in direct contact with wastewater that had been stored in a reservoir (quality \(10^4\) FC/100ml) had a four-fold increase in seroresponse to Human Norwalk-like virus / Mexico infection after allowance for confounding factors.

4.2.4 Overall.

There is evidence to suggest that direct contact with untreated wastewater can lead to increased helminth infection (mainly *Ascaris* infection), and that this effect is more pronounced in children than adults. There is some evidence that *Ascaris* infection related to direct contact with wastewater can be reduced when wastewater is partially treated before use, but the effect depends on the extent of treatment. Treatment may need to be below the level of \(1\) egg/litre where children are exposed.

When the results of studies assessing the risk of enteric infection among adults exposed to wastewater aerosols from sprinkler irrigation of treated wastewater are taken together (Figure 1) there is no significant difference between the effect of exposure to wastewater of different qualities, in the range shown. Comparisons are hindered by differences in the qualities of the studies compared (methodologically), and the inclusion of studies with both disease outcomes and serological outcomes. If just the studies with disease outcomes are reviewed, there is an indication that when sprinkler irrigation is used there was an increased risk of infection when the quality of the wastewater was \(10^6\) FC/100ml, but very little increased risk of infection when the water quality was \(10^4-5\) FC/100ml or less.

When the results of studies assessing the risk of enteric infection related to direct contact with wastewater are taken together (Figure 2), there is no clear exposure-response effect and no significant difference between the effect of exposure to wastewater of different qualities in the range shown. The higher risks related to exposure in one study where the water quality was \(10^4\) FC/100ml may be due to the increased wastewater contact occurring during flood irrigation, greater effects in the dry season, and detailed measurement of individual wastewater exposure.

4.3 Risks to consumers of vegetables and to farming families from use of excreta to fertilize crops

In areas where human excreta is used as a fertilizer for crops, a high prevalence and intensity of *Ascaris* infection has often been reported (for example, in Iran and China); hookworm infection is also highly prevalent in wetter climates where excreta is used (for example, Vietnam and Southern China). Blum and Feachem include description of some of the older, descriptive studies of the prevalence of helminth infections in areas where excreta is used as fertilizer.

The risks to consumers and farm workers exposed to untreated or treated excreta used as fertilizer for crops are shown in Table 9. Cross-sectional studies in Germany have indicated that school children living in an urban area where vegetables were fertilized with excreta, and those living in a rural area where human excreta (together with animal manure) was used to fertilize vegetables were at increased risk of *Ascaris* infection compared with school children living in a sewered urban area. A second study in Germany indicated a higher relative risk associated with use of excreta as garden fertilizer. However, these results are suggestive only, as in both studies the control groups were not comparable with the exposed groups, and the type and extent of exposure of the study groups was not ascertained.
Figure 1  Relative risk of enteric infection among adults in aerosol contact with wastewater of varying microbiological quality (comparisons as in Tables 7 + 8)

Figure 2  Relative risk of enteric infection among individuals in direct contact with wastewater of varying microbiological quality (comparisons as in Tables 7 + 8)
Studies on the effectiveness of treatment of excreta before use as fertilizer have been done in Japan\textsuperscript{37,38}. In an experimental study, positive conversion to \textit{Ascaris} and hookworm after chemotherapy was measured in a rural farming population exposed to nightsoil treated with ovicide over a period of 8 months (Na-nitrite and calcium superphosphate mixture) and a control population (from the same area) exposed to untreated nightsoil\textsuperscript{37}. The ovicide was applied monthly to the tanks used for nightsoil storage and in the nightsoil reservoir of the toilet in the farmer’s house. The type of crops fertilized with nightsoil and the type of exposure of the study population is not specified, so it is not clear whether exposure was through direct contact with the treated nightsoil, or through consumption of nightsoil-fertilized crops or both. Exposure to treated nightsoil was significantly associated with a 34% reduction in \textit{Ascaris} infection and a 45% reduction in hookworm infection compared with the exposure to untreated nightsoil. Baseline prevalence rates in the study groups were similar. The crude chemical mixture was more effective than a commercial preparation in the case of \textit{Ascaris} infection. The authors report that the lowest incidence of infection was found in the families which used nightsoil tanks, which may facilitate the treatment of faeces with chemicals.

In another experimental study\textsuperscript{38}, the effect of the use of thiabendazole to treat excreta before use was compared in groups where egg excretion continued (no chemotherapy was given to the study group) and where egg excretion was reduced by chemotherapy; a control group was given chemotherapy alone and used untreated excreta. The thiabendazole was applied monthly to the nightsoil reservoir in each house, and nightsoil was not used as fertilizer until at least 7 days exposure to the ovicide. There were pre-existing baseline differences in prevalence rates. Before-after differences were therefore analysed in each study group separately (Table 9). Treatment of nightsoil was significantly associated with a 37% reduction in \textit{Ascaris} infection in both treatment groups; chemotherapy alone did not result in a significant reduction in infection. Treatment of nightsoil was also significantly associated with a 27% reduction in \textit{Trichuris} infection (in a separate trial). Exposure was mainly through consumption of vegetables fertilized with nightsoil as there was no relationship between farm work and \textit{Ascaris} or \textit{Trichuris} infection. In this study, the sample size was too small to detect a significant reduction in hookworm infection related to farm work.

A study was reported from Vietnam on the traditional treatment of faeces before use as fertilizer\textsuperscript{39}. Women helped prepare and distribute the faeces on the crops. Most used fresh faeces but some used wet, dry or composted faeces for fertilizer. Dry faeces mixed with ash were distributed with a shovel or by hand, whereas wet faeces were mixed with water and poured onto the plants using dippers or buckets. Treatment of faeces consisted of mixing dry faeces with ash and putting the mixture in a pit along with coconut and banana leaves and ‘dirt’. Most families used the faeces before it had been stored for 4 months, the time needed for it to be considered biologically safe (Hanoi Medical School, 1994: unpublished observations). Women who reported using fresh faeces as fertilizer had significantly higher hookworm egg counts (Mann-Whitney U test, \(p<0.05\)) than women who used treated faeces or who did not use human faeces as fertilizer. Since the result was not reported separately for those who used treated faeces, nothing can be concluded about the effectiveness of treatment of faeces on hookworm infection. There is some indication, however, from the data presented that treatment of faeces may reduce the number of women with higher intensity infections.

5. Discussion

5.1 Limitations of literature

There are numerous water and excreta-related parasitic, bacterial and viral infections which could be transmitted through wastewater reuse, yet often no epidemiological studies have tested whether
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome</th>
<th>Excreta quality</th>
<th>Population group</th>
<th>Prevalence of infection or re-infection after treatment (%)</th>
<th>Relative risk</th>
<th>Study group and comparison</th>
</tr>
</thead>
</table>
| Anders34 (1952) | Ascaris | Overflowing septic tank contents and excreta composted with animal manure | School children | (i) 14.3 vs 2.9  
(ii) 6.7 vs 2.9 | 4.9^a  
2.3 | School children (i) in urban area where vegetables fertilized with overflowing septic tank contents vs in sewered urban area  
(ii) in rural area where human faeces composted with animal manure or applied at ‘appropriate’ time to vegetables vs in sewered urban area |
| Kreuz35 (1955) citing Harmsen36 (1953) | Ascaris | Untreated | Farming population | 52 vs 0 | 52.0^b | Families using excreta as garden fertilizer vs families using animal manure as garden fertilizer |
| Kozai37(1962) | Ascaris (+ve conversion after chemotherapy) | Ovicide treated | Farming population | (i) 27.4 vs 41.5  
(ii) 35.9 vs 41.5 | 0.66 (0.51-0.86)^**  
0.86 (0.69-1.07) | (i) Ovicide-treated nightsoil vs untreated nightsoil  
(ii) Ovicide-treated nightsoil (commercial preparation) vs untreated nightsoil |
| Kutsumi38 (1969) | (i) Ascaris (prevalence)  
(ii) Trichuris (prevalence) | Ovicide treated | Farming population | A 11.0 vs 17.5  
B 21.0 vs 33.1  
C 14.6 vs 11.6  
(ii) 47.1 vs 65.0 | 0.63 (0.40-0.98)^*  
0.63 (0.44-0.92)^*  
0.79 (0.53-1.18)^*  
0.73 (0.64-0.82)^* | (i) A After nightsoil treatment with ovicide plus chemotherapy vs before treatment  
B After nightsoil treatment with ovicide vs before treatment  
C Chemotherapy alone  
(ii) After nightsoil treatment plus ovicide vs before treatment |
| Kozai37(1962) | Hookworm (+ve conversion after chemotherapy) | Ovicide treated | Farming population | (i) 17.7 vs 32.2  
(ii) 17.4 vs 32.2 | 0.55 (0.36-0.81)^**  
0.54 (0.36-0.81)^** | (i) Ovicide-treated nightsoil vs untreated nightsoil  
(ii) Ovicide-treated nightsoil (commercial preparation) vs untreated nightsoil |
| Kutsumi38 (1969) | Hookworm (+ve conversion after chemotherapy) | Ovicide treated | Adults | 7.1 vs 12.5 | 0.56 (0.27-1.16) | Families using ovicide-treated nightsoil vs untreated nightsoil |
| Humphries39 (1997) | Hookworm | Treatment with ash and storage | Adult women | P<0.05 | Egg counts in women using fresh faeces vs women using treated faeces or not using faeces as fertilizer |

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*a* Comparison is exposed vs unexposed for untreated excreta use; comparison is treated vs untreated excreta or after vs before treatment for treated excreta  
*b* crude relative risk calculated from prevalence or incidence data reported  
*c* relative risk and 95% confidence interval calculated from prevalence or incidence rates and population data reported

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they are associated with wastewater reuse or not, and particularly, few studies have tested the
effect of treatment of the wastewater before use. This is especially the case for risks to consume of
uncooked vegetables that have been irrigated with wastewater, where there is a paucity of studies
related to bacterial and viral infections, and very few studies of the effect of treatment of
wastewater (Table 1).

Many of the early epidemiological studies were descriptive studies, and cannot provide accurate
information on the association between wastewater reuse and health outcomes. They have been
included here to provide supporting information in cases where there are few analytical studies of
the relationship in question. All the studies of wastewater reuse are observational; the nature of the
exposure and of wastewater treatment and the practicalities involved make it difficult to use
experimental studies. The main factors that affect the validity of the reported studies and their
usefulness in the setting of guidelines are listed below (see comment columns of Tables 1, 2, 3
and 4):

a) Bias due to the misclassification of exposure. Exposure is classified in two ways, according to
(I) the occurrence of human contact with the wastewater through contact or consumption, and
(II) the microbiological quality of the wastewater.
(I) Where exposure was classified at a group and not at an individual level,
misclassification of exposure is likely to have occurred. Individuals who lived in a
wastewater irrigated area but were not actually exposed (i.e. did not consume
uncooked wastewater irrigated vegetables, or did not have direct contact with
wastewater) were included within the exposed group. This would lead to an
underestimation of the effect of exposure. The level at which exposure was measured
in each study is given in Tables 1, 2, 3 and 4. Bias due to misclassification of
exposure also occurred in a retrospective study where records of the use of
wastewater on particular communities were incorrect, leading to an overestimation of
the effect of exposure26.
(II) Inadequate measurement of water quality can also lead to misclassification of
exposure. In most studies, either no measure of water quality is given or a mean value
is given, with no measure of the variation in water quality. In a few prospective cohort
studies, regular measurements of water quality are reported for the period of the study
allowing a seasonal mean and range of water quality to be given15,35. The indicators
used are total and faecal coliforms per 100ml and nematode eggs per litre. Where
seasonal means alone are used, these may give artificially low threshold values for
increased risk47.

b) Bias due to the misclassification of disease. There is potential for recall bias on studies of
self-reported disease where individuals with a particular exposure may recall their experiences
differently from those who are not exposed. This could have occurred in the studies of
symptomatic diarrhoeal disease (Table 5 and 7). Studies using clinic records, particularly
retrospective cohort studies (where nothing can be done to affect the quality of reporting) may
suffer from misclassification of disease due to differences in the quality of reporting in
different communities. This can lead to an under or overestimation of the effect.

c) Lack of evaluation of the presence of a valid statistical association between the exposure and
the outcome. Many studies either do not report any significance testing, or report insufficient
information on the significance test carried out. Since sample sizes are low in many studies,
reporting of confidence intervals and P values is particularly important. Where they have not
been reported, we have calculated (crude) relative risks and 95% confidence intervals.

d) Lack of control for potential confounders. Where other risk factors for the health outcome are
not measured and controlled for in the statistical analysis, this can lead to an over or
underestimation of the risk of wastewater exposure. In studies in USA\textsuperscript{29}, the risk of clinical illness related to aerosol exposure to treated wastewater did not remain after the association was controlled for eating at local restaurants. There are very few studies that have measured and controlled for potential confounding factors, and it is likely that some of the earlier estimates of the association between wastewater exposure and disease are overestimates because of this.

e) Selection of limited study population. In many studies, the effect of age on wastewater exposure is not explored. Early studies of the risks to farm workers concentrated on adult populations, whereas recent studies have included children (who often work in or play near wastewater irrigated fields), who may not have developed immunity to the infection in question.

Table 10 \textit{The 1989 WHO guidelines for the use of treated wastewater in agriculture}\textsuperscript{a}

<table>
<thead>
<tr>
<th>Category</th>
<th>Reuse conditions</th>
<th>Exposed group</th>
<th>Intestinal nematode\textsuperscript{b} \hspace{1cm} (arithmetic mean no. eggs per litre)</th>
<th>Faecal coliforms \hspace{1cm} (geometric mean no. per 100ml)\textsuperscript{c}</th>
<th>Wastewater treatment expected to achieve the required microbiological guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Irrigation of crops likely to be eaten uncooked, sports fields, public parks\textsuperscript{d}</td>
<td>Workers, consumers, public</td>
<td>\textless{} 1</td>
<td>\textless{} 1000</td>
<td>A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment</td>
</tr>
<tr>
<td>B</td>
<td>Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees\textsuperscript{e}</td>
<td>Workers</td>
<td>\textless{}1</td>
<td>No standard recommended</td>
<td>Retention in stabilization ponds for 8-10 days or equivalent helminth and faecal coliform removal</td>
</tr>
<tr>
<td>C</td>
<td>Localized irrigation of crops in category B if exposure to workers and the public does not occur</td>
<td>None</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Pretreatment as required by irrigation technology, but not less than primary sedimentation</td>
</tr>
</tbody>
</table>

\textsuperscript{a} In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.

\textsuperscript{b} \textit{Ascaris} and \textit{Trichuris} species and hookworms.

\textsuperscript{c} During the irrigation period.

\textsuperscript{d} A more stringent guideline (\textless{} 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

\textsuperscript{e} In the case of fruit trees, irrigation should cease two weeks before fruit is picket, and no fruit should be picked off the ground. Sprinkler irrigation should be used.

5.2 \textit{Review of findings and application to derivation of guidelines for wastewater use}

The findings will be discussed in relation to the categories of exposure used in WHO (1989) guidelines (Table 10). The results of studies with least methodological weaknesses will be given precedence over those with significant weaknesses. Most of the studies are cross-sectional or prospective cohort studies. Where the latter exist, their results are given precedence since there is a greater likelihood the relationship is causal due to the time sequence i.e. where the exposure precedes the outcome of interest. Where studies provided adequate data on the characteristics of the exposed and control groups and controlled the measure of association for potential
confounding factors, their results are given precedence over studies where this did not occur. For these studies, information on attributable risks is given in Tables 11, 12 and 13 and taken into account in the derivation of guidelines. The calculation of attributable risks assumes causation has been shown in the relationship between exposure and the infection or disease outcome. The results should be interpreted with some caution, as in some studies causation has not been shown (for example, the cross-sectional studies) and in others, control for confounding may not be complete. In addition, attributable risks are specific to the study site, and cannot be generalised to other sites where the presence of different risk factors may alter the situation.

5.2.1 Category A Unrestricted irrigation

There is convincing evidence that use of untreated wastewater to irrigate vegetables leads to increased helminth infection (mainly *Ascaris* infection), bacterial infections (typhoid, cholera, *Helicobacter pylori*) and symptomatic diarrhoeal disease in consumers.

It is clear that wastewater treatment markedly reduces the risk of helminth infections (particularly *Ascaris* infection) related to consumption of wastewater irrigated crops (Figure 1), but the extent of treatment needed to remove the risk is not fully clear. Evidence from Germany of the effectiveness of sedimentation and biological oxidation in removing the risks of *Ascaris* infection is suggestive only, as this was an ecological study and causation cannot be inferred. The studies of use of wastewater stored in reservoirs from Mexico\(^{12,13}\) are limited by the fact that stratum-specific estimates of the effect of consumption (in untreated and treated wastewater strata) do not control for confounding factors (the studies were designed to assess the effect of direct contact with wastewater through farm work, consequently more detailed analysis of the data on consumption was not carried out). The best estimate available, from the prospective cohort study\(^{13}\) suggests there are risks associated with use of untreated wastewater, and there may be similar effects when treated wastewater of quality \(\leq 1\) nematode egg per litre is used, but this has not been shown statistically (due to the small numbers involved). The proportion of *Ascaris* infection in the study population that was attributed to consumption of uncooked vegetables irrigated with wastewater was 53% for the under 15’s and 35% for adults. There is a suggestion, therefore that treating the wastewater to the level of \(\leq 1\) nematode egg per litre may not be sufficient (especially in an area where flood irrigation is used and field vegetables are eaten by the local population), but this has only been shown in one study.

The studies from Mexico\(^{12,15}\) provide the only epidemiological evidence available of the effect of consuming vegetables irrigated with partially-treated wastewater on diarrhoeal disease. Evidence from the second study\(^{15}\) takes precedence over evidence from the first study\(^{12}\) as this was designed to gain more extensive, and more accurate data on vegetable consumption and water quality; yet, the results were of the same order of magnitude. In the second study, the wastewater had been retained in one storage reservoir before release into the river and use in irrigation. The quality of the water at the irrigation site was \(3x10^4\) FC/100ml, which was stable over the year (daily sampling). The results indicated a two-fold or greater excess of symptomatic diarrhoeal disease in children and adults consuming uncooked onions in the dry season. Over 50% of the diarrhoeal disease in the study population who ate onions (which was over half over the study group) was attributable to consumption of onions (Table 11), such that over 25% of all diarrhoea in the adults and young children in the study population in the dry season was attributable to wastewater irrigation of vegetables. Consumption of green tomatoes was associated with a two-fold increase in serological response to Human Norwalk-like Virus / Mexico. This indicates the potential for transmission of infection, but does not necessarily indicate that clinical illness would occur. In this population, however, as the proportion who ate green tomatoes was low, the population attributable fraction was only 9%. Both effects were observed after controlling for many potential confounding factors.
The results suggest that the risk of infection is significant when WHO guidelines of 1000 FC/100ml are exceeded by a factor of 10. Classification of exposure was done very carefully to avoid misclassification as far as possible, using measures of recent consumption (frequency of consumption of individual vegetables and total consumption of uncooked vegetables in the last 7 days) and ‘usual’ consumption of individual vegetables. In a rural community where there are other routes of transmission the threshold level is below $10^4$ FC/100ml. There are no epidemiological data available to indicate whether the threshold level might be similar or lower in countries with less concurrent routes of transmission.

Table 11  Studies of consumer risk – attributable risk and population attributable fraction

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome and outcome measure</th>
<th>Wastewater quality</th>
<th>Age group (years)</th>
<th>Prevalence of outcome measure (%)</th>
<th>Attributable risk (population attributable fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peasey13 (2000)</td>
<td>Ascaris (positive conversion after chemotherapy)</td>
<td>Untreated and treated (one reservoir &lt;1 nematode egg per litre)</td>
<td>&lt; 15 &gt; 15</td>
<td>41.1 vs 16.4 22.4 vs 8.8</td>
<td>24.7 (53.4) 13.6 (34.8)</td>
</tr>
<tr>
<td>Hopkins14 (1993)</td>
<td>Helicobacter pylori (seroprevalence)</td>
<td>Untreated</td>
<td>&lt;35</td>
<td>38 vs 9</td>
<td>29.0 (74.0)</td>
</tr>
<tr>
<td>Cifuentes12 (1998)</td>
<td>Diarrhoeal disease (2-weekly prevalence)</td>
<td>Untreated and treated</td>
<td>5+</td>
<td>19.0 vs 10.0</td>
<td>9.0 (3.8)</td>
</tr>
<tr>
<td>Blumenthal15 (2001a)</td>
<td>Diarrhoeal disease (weekly prevalence)</td>
<td>Treated (storage reservoirs) $10^4$ FC/100ml</td>
<td>1-4 15+</td>
<td>&lt;1/wk 1/wk +</td>
<td>2.6 8.2 2.4 6.7</td>
</tr>
<tr>
<td>Blumenthal15 (2001a)</td>
<td>Human Norwalk-like virus/Mexico (seroresponse)</td>
<td>Treated (storage reservoirs) $10^4$ FC/100ml</td>
<td>5-14 0/2wks 1/2wks+</td>
<td>13.6 23.0</td>
<td>9.4 (8.7)</td>
</tr>
</tbody>
</table>

* studies are only included where a significant association between exposure and outcome measure has been shown after allowance for confounding factors (see Table 5)

5.2.2  Category B Restricted irrigation

Studies of the risks of Ascaris infection to farm workers and their families related to use of treated wastewater12,13,20 suggest that the threshold level for increased prevalence of infection is below 1 nematode egg per litre (Table 6). There is some evidence to suggest that the threshold may be above 1 nematode egg per litre for adults, but below 1 nematode egg per litre for children. Data from one study20 appears to go against the trend above; however, this is due to the data on 5-14 year olds and adults being combined in a single measure for 5+ years. The risk reported is due to the effect in 5-14 year olds rather than in adults (shown in the prevalence data). In the studies on which these conclusion are based12,13,20 a water quality of <1 nematode egg per litre was achieved through retention in 1 reservoir for a minimum of one month over the year whereas retention in two reservoirs acting in series for a period of 1-2 months in each reservoir improved the quality such that no nematode eggs were detected. The low infectious dose for Ascaris infection, and the persistence of eggs in the environment (such that eggs could accumulate on the soil and result in increasing exposure) could explain the low threshold level. However, it may be artificially low, due to the sampling procedure adopted in large irrigation canals, as eggs may be less frequent at the sides of the canals (accessible for sampling) than in the centre and bottom of the canals.

The proportion of Ascaris infection in the population attributable to exposure (population attributable fraction) appeared to be very high (Table 12) when wastewater was either untreated or only treated in one reservoir20 (treatment achieving 2 log nematode egg removal). The estimate of the population attributable fraction was affected by the comparison group used. It was greater
when the comparison group was a separate population, that is, when populations using wastewater were compared with those practising rain-fed agriculture\(^{20}\). This provides a measure of the effect on *Ascaris* infection of introducing wastewater into an area, but may be affected by residual confounding that has not been allowed for in the analysis. When an internal comparison group was used, the population attributable fraction was much lower, that is, when persons with contact with wastewater from one reservoir were compared with those in the same area but with no contact with wastewater (so reducing confounding effects related to differences between areas)\(^{13}\). This measures the proportion of infection in the exposed area than could be prevented through appropriate intervention. In this case, the risk to the under 15’s was related to play, and the proportion of *Ascaris* infection in the population attributable to exposure was only 10% (as only a small number had contact with treated wastewater through play). When the wastewater was further treated in a second storage reservoir (no nematode eggs detected) the proportion of disease in the population attributable to exposure was still 36%, but the attributable risk itself (that is, the excess prevalence in the exposed group compared with the control group) was very low (0.8%)\(^{12}\). This suggests that treatment to the level of \(\leq 1\) nematode egg per litre (through one reservoir or treatment) combined with measures to restrict the contact of children with wastewater through play could be adequate, if treatment to below the level of \(\leq 1\) nematode egg per litre could not be provided.

Table 12: Studies of risks of *Ascaris* infection in farm workers and their families\(^{a}\) – attributable risk (excess prevalence) and population attributable fraction

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome</th>
<th>Wastewater quality</th>
<th>Age group (years)</th>
<th>Prevalence of infection (% (exposed vs control group))</th>
<th>Attributable risk (population attributable fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blumenthal(^{20}) (2001b)</td>
<td>Ascaris (prevalence)</td>
<td>(i) Untreated (ii) Treated (one storage reservoir)</td>
<td>0-4</td>
<td>(i) 10.0 vs 0.6 (ii) 11.8 vs 0.6</td>
<td>9.4 (88.9) 11.2 (90.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;1 nematode egg/litre</td>
<td>5+</td>
<td>(i) 7.2 vs 0.4 (ii) 4.6 vs 0.4</td>
<td>6.8 (89.7) 4.2 (84.6)</td>
</tr>
<tr>
<td>Cifuentes(^{12}) (1998)</td>
<td>Ascaris (prevalence)</td>
<td>(i) Untreated (ii) Treated (two storage reservoirs)</td>
<td>0 nematode egg/litre</td>
<td>0-4</td>
<td>(i) 13.7 vs 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5+</td>
<td>(i) 9.1 vs 0.7 (ii) 1.5 vs 0.7</td>
</tr>
<tr>
<td>Peasey(^{13}) (2000)</td>
<td>Ascaris (positive conversion after chemotherapy)</td>
<td>(i) Untreated (ii) Treated (one storage reservoir)</td>
<td>&lt;15</td>
<td>(ii) 44.1 vs 30.0</td>
<td>14.1 (9.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;1 nematode egg/litre</td>
<td>&gt;15</td>
<td>(i) 39.3 vs 9.0</td>
<td>30.3 (33.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>male</td>
<td>(i) 37.5 vs 15.6</td>
<td>21.9 (12.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>female</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) studies are only included where a significant association between exposure and outcome measure has been shown after allowance for confounding factors (see Table 6)

Studies of the risks of enteric viral and bacterial infections related to use of treated wastewater (both from serological studies and studies of reported enteric infections) suggest that when sprinkler irrigation is used and the population is exposed to wastewater aerosols, the threshold level was between \(10^4\)-\(10^5\) and \(10^6\) TC/100ml in kibbutzim in Israel (where communal living occurs and immunity develops to viral infection circulating in the community), and was between \(10^3\)-\(10^4\) and \(10^6\) FC/100ml in a rural community in USA exposed to treated wastewater from a nearby town. A threshold level of around \(10^5\) FC/100ml could apply to both types of situation (Figure 4), though it is possible that the threshold may be slightly lower in situations where less communal living occurs. The proportion of infection\(^{31}\) or disease\(^{24}\) in the study population that could be attributed to exposure to wastewater aerosols (\(10^6\) FC/100ml) was 27% (all ages) and 31% (young children) respectively (Table 13). In such study populations, improvement of the wastewater quality to \(10^4\)-\(10^5\) FC/100ml should remove such risks.
Studies of the risks of symptomatic diarrhoeal disease and enteric viral infections related to direct contact with treated wastewater, through farm work (adults and children) or play (children) suggest that when flood or furrow irrigation occurs the threshold level is below $10^4$ FC/100ml for children. For adults, the threshold level for symptomatic diarrhoeal disease was $10^5$ FC/100ml, but the threshold level for transmission of Human Norwalk-like Virus/Mexico (as indicated by an increased level of seroresponse) was below $10^4$ FC/100ml where high levels of contact occurred, even in a rural area where there were many other transmission routes for this virus. This was probably not related to an increase in disease in the study area, but could conceivably lead to disease in a non-endemic area. When the water quality was $10^4$ FC/100ml, the proportion of diarrhoeal disease in the population of school-aged children that was attributable to exposure to this water was around 20% (Table 13), and there was no excess risk in other age groups. Protection measures targeted at this age group could be considered as an alternative to improving water quality to $10^3$ FC/100ml.

5.3 Review of findings and application to derivation of guidelines for excreta use

Since 1985, there has been very little new epidemiological research reported in the searchable literature on the health effects of the use of excreta in agriculture. Only one controlled epidemiological study was located where the effect of use of excreta as fertilizer was compared in exposed and comparison groups (Table 9). This study showed that use of fresh faeces as fertilizer was associated with increased intensity of hookworm infection in exposed women, when compared to women who use treated faeces or who did not use excreta as fertilizer. Unfortunately there was no comparison of those who used treated faeces with those who did not use excreta (possibly due to sample size effects). However, as the treated faeces were stored for less than 4 months before use, it is unlikely that it was effective in reducing hookworm infection.

The evidence does not show that there is any need to revise the guidelines for excreta use in agriculture reported in 1989 except possibly in relation to modifications to the wastewater guidelines, on which they are based (see below). Advances in excreta treatment may mean that there are increased choices in the methods available for the treatment of excreta before use. Treatment with ovicide could be considered (as occurs in parts of China), alongside consideration of technologies for excreta storage and composting or thermophilic digestion.

5.4 Adequacy of the information for the derivation of guidelines for wastewater use

The epidemiological information reviewed provides a better basis for the derivation of guidelines for wastewater use in agriculture than existed for the WHO guidelines published in 1973 and 1989. There are at least one or two methodologically sound studies available (good classification of exposure and disease, allowance for confounding factors, statistical testing) which relate to the guideline values in each category of the table of microbiological guidelines given in the 1989 WHO guidelines (Table 10). The database of epidemiological information is not as good as the one on the health effects of recreational water where sufficient studies have been done to allow thresholds and dose-response effects to be investigated further.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Health outcome and outcome measure</th>
<th>Wastewater quality</th>
<th>Age group (years)</th>
<th>Prevalence or incidence of outcome measure</th>
<th>Attributable risk (population attributable fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fattal24 (1986) Enteric disease (Incidence per 100 person-years)</td>
<td>Treated Stabilization ponds 5-10 day detention $10^{6.8}$ FC/100ml</td>
<td>0-4</td>
<td>51.8 vs 27.4</td>
<td>24.4 (30.8)</td>
<td></td>
</tr>
<tr>
<td>Camann31 (1986) Enteric viruses Seroconversion incidence densities per 100 person years</td>
<td>Treated (i) Irrigation vs baseline (ii) High exposure: irrigation vs baseline</td>
<td>All ages (i) 5.4 vs 2.6 (ii) 8.3 vs 1.3</td>
<td>2.8 (26.7)</td>
<td>7.0 (62.0)</td>
<td></td>
</tr>
<tr>
<td>Blumenthal20 (2001b) Diarrhoeal disease (2-weekly prevalence)</td>
<td>(i) Untreated and (ii) Treated (one storage reservoir) $10^3$ FC/100ml</td>
<td>0-4 5+</td>
<td>(i) 19.4 vs 13.6 (ii) 7.1 vs 5.9 (iii) 8.1 vs 5.9</td>
<td>5.8 (15.0)</td>
<td>1.2 (6.3)</td>
</tr>
<tr>
<td>Blumenthal15 (2001a) Diarrhoeal disease (weekly prevalence)</td>
<td>Treated $10^3$FC/100ml (i) High vs no contact (rainy season) (ii) Contact vs no contact (dry season)</td>
<td>5-14</td>
<td>(i) 11.0 vs 4.0 (ii) 7.4 vs 3.2</td>
<td>7.0 (18.4)</td>
<td>4.2 (23.0)</td>
</tr>
<tr>
<td>Blumenthal15 (2001a) Norwalk-like virus Mexico (% seroresponse)</td>
<td>Treated $10^3$FC/100ml</td>
<td>&gt;15 years</td>
<td>33.3 vs 11.4</td>
<td>21.9 (28.3)</td>
<td></td>
</tr>
</tbody>
</table>

* studies are only included where a significant association between exposure and outcome measure has been shown after allowance for confounding factors (see Tables 7 and 8)

Modifications to the microbiological guidelines could be proposed on the basis of the epidemiological studies reviewed, but they would be more convincing if supported by other data. Modified guideline values were recently proposed, based on the available epidemiological data and supported by data from microbiological studies and risk assessment studies (Table 14). Wastewater treatment techniques suitable for achieving the guideline values and other health protection measures are described in a recent report. The guidelines were proposed so that there should be no measurable excess cases in the exposed population, as adopted by WHO (1989) and supported by studies in which a model-generated estimated risk was compared with a defined acceptable risk. The modified guidelines for restricted irrigation were based on a larger number of studies than were available to support the guidelines for unrestricted irrigation (as in the current report). For the latter, evidence from risk assessment studies was particularly important to support the proposal that there is no evidence to warrant a change in the faecal coliform guideline value for unrestricted irrigation. Treatment recommendations take into account that stable systems are preferable to systems which provide less consistent quality effluent, where short periods of low quality water could lead to transmission of infection.

The proposed revisions to the guidelines may appear too restrictive, when consideration is given to the proportion of disease in the population that is attributable to exposure, and could therefore be averted if water quality improvements were made (the preventable fraction). When guidelines are set, a decision needs to be made on when the preventable fraction is sufficient to warrant large and costly improvements in the quality of all wastewater destined for reuse. For example, if 5% of disease in the population can be averted by treating wastewater to $10^3$ FC/100ml, is that sufficient to warrant the cost, or does the figure need to rise to 10, or 20%? Data on costs per case averted can help in the making of such decisions, as has been tried based on risk assessment studies. Such decisions are best made at a country level, where consideration can be given to the importance of other risk factors, and the feasibility and cost of the available intervention.
measures. However, it may be necessary to agree on an appropriate figure at an ‘international’ level to aid in the setting of international guidelines. In the Water Decade (1981-90) it was suggested that interventions acting on causes of less than 5% of diarrhoeal disease should not be prioritised55.

The microbiological guidelines are based on situations where wastewater treatment is the main intervention being adopted. However, where population attributable fractions are low, but risks to particular groups are high (e.g. for enteric disease in farm workers and their school aged children) use of partial wastewater treatment and the targeting of other health protection measures to the specific groups could be considered (see integrating health protection measures in WHO (1989) guidelines). For example, Categories B2 and B3 could theoretically include a faecal coliform guideline of $\leq 10^5$ FC/100ml and a nematode egg guideline of $\leq 1$ nematode egg per litre (as in category B1) if additional health promotion measures were taken to help adult farm workers and children to improve hygiene measures post-wastewater contact. There is some epidemiological evidence of the importance of good hygiene19, but little evidence that current promotional techniques in environmental health can have an impact on behaviour change56. There is new evidence that specific protective hygiene behaviours are more easily and effectively modified for social and cultural reasons, rather than through fear of illness57.

Where recommendations are based on only one study and circumstances may not be representative, then taking a less restrictive approach could be considered. For example, for unrestricted irrigation (Category A), the nematode egg guideline could be set at $\leq 1$ nematode egg per litre, to cover circumstances where vegetables are grown in hot and dry conditions (in tropical countries or in greenhouses) and may only need to be more restrictive, at 0.1 nematode egg per litre in circumstances where surface irrigation is used and field vegetables are eaten by the local population.

The setting of country standards will need to take into account the particular epidemiological scenario in the country, as well as social, cultural and economic considerations. For example, standards for wastewater reuse may need to be less restrictive in counties with a high level of endemic gastro-intestinal disease, where there are many other important transmission routes, and immunity to some infections (for example, viral infections) is acquired early in life. Standards may need to be stricter in countries with lower levels of endemic disease where wastewater reuse could be a major transmission route for gastro-intestinal disease, and where epidemics of gastro-intestinal disease are of particular concern for non-immune sectors of the population.

There are insufficient new data on the effect of excreta use in agriculture to warrant any changes to the guidelines for excreta use in agriculture7.

5.5 Water quality guidelines or health targets

In the past, the emphasis has been on setting water quality guidelines (or national standards) and then assessing whether the guidelines (or national standards) have been met by monitoring water quality. More recently, a new health-based approach has been advocated, where health targets would be set based on an assessment of risk and a measure of what is an ‘acceptable risk’56. Water quality targets would then be based on the health targets, and would be achieved through the use of a risk management process. A reference level of acceptable risk would be defined using a common exchange unit, such as the Disability Adjusted Life Years (DALY’s) that could account for acute, delayed and chronic effects.
<table>
<thead>
<tr>
<th>Category</th>
<th>Reuse Conditions</th>
<th>Exposed group</th>
<th>Irrigation technique</th>
<th>Intestinal nematodes (arithmetic mean no of eggs per litre)</th>
<th>Faecal coliforms (geometric mean no per 100ml)</th>
<th>Wastewater treatment expected to achieve required microbiological quality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Unrestricted irrigation</td>
<td>Workers, consumers, public</td>
<td>Any</td>
<td>≤0.1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>≤ 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Well designed series of waste stabilization ponds (WSP), sequential batch-fed wastewater storage and treatment reservoirs (WSTR) or equivalent treatment (e.g. conventional secondary treatment supplemented by either polishing ponds or filtration and disinfection)</td>
</tr>
<tr>
<td>A1 Vegetable and salad crops eaten uncooked, sports fields, public parks&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Restricted irrigation</td>
<td>B1 Workers (but no children &lt;15 years), nearby communities</td>
<td>(a) Spray/sprinkler</td>
<td>≤ 1</td>
<td>≤ 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Retention in WSP series inc. one maturation pond or in sequential WSTR or equivalent treatment (e.g. conventional secondary treatment supplemented by either polishing ponds or filtration)</td>
</tr>
<tr>
<td>B2 As B1</td>
<td>(b) Flood/furrow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>As for Category A</td>
</tr>
<tr>
<td>B3 Workers including children &lt; 15 years, nearby communities</td>
<td>Any</td>
<td>≤0.1</td>
<td>≤ 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>As for Category A</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Localised irrigation of crops in category B if exposure of workers and the public does not occur</td>
<td>None</td>
<td>Trickle, drip or bubbler</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Pretreatment as required by the irrigation technology, but not less than primary sedimentation</td>
</tr>
</tbody>
</table>

<sup>a</sup> In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.

<sup>b</sup> Ascaris and Trichuris species and hookworms; the guideline is also intended to protect against risks from parasitic protozoa.

<sup>c</sup> During the irrigation season (if the wastewater is treated in WSP or WSTR which have been designed to achieve these egg numbers, then routine effluent quality monitoring is not required).

<sup>d</sup> Retention in WSP series inc. one maturation pond or in sequential WSTR or equivalent treatment (e.g. conventional secondary treatment supplemented by either polishing ponds or filtration).

<sup>e</sup> A more stringent guideline (≤ 200 fecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.

<sup>f</sup> During the irrigation season (faecal coliform counts should preferably be done weekly, but at least monthly).

<sup>g</sup> This guideline can be increased to ≤1 egg per litre if (i) conditions are hot and dry and surface irrigation is not used, or (ii) if wastewater treatment is supplemented with anthelmintic chemotherapy campaigns in areas of wastewater re-use.

<sup>h</sup> In the case of fruit trees, irrigation should cease two weeks before fruit is picked and no fruit should be picked off the ground. Spray/sprinkler irrigation should not be used.
This approach can be used for the setting of national standards, taking into account national views on acceptable risks, and the diseases of importance in that country. Using the epidemiological data available, this approach could conceivably be used in the setting of international guidelines. Two approaches could be considered

(i) the level of disease in the ‘control’ population not exposed to wastewater could be considered the ‘acceptable risk’ level, and no statistically significant increase in disease above this level would be accepted. Water quality targets would be set at the level where studies showed that no statistically significant increase in disease occurred. This is the approach taken in making the proposals in Table 14. It should be noted, however, that the sample size in most studies is not sufficient to detect relative risks lower than about 1.5 at a statistically significant level, when infection in exposed and control groups are compared, such that this approach may involve the acceptance of around a 1.5 fold increase in risk in the exposed population.

(ii) the measure of risk used to define an ‘acceptable risk’ could be an attributable risk expressed as a proportion (specifically, the population attributable fraction, PAF) and not an absolute measure of risk, measured in terms of morbidity or DALY’s. So, an acceptable population attributable fraction for wastewater use could be set at, say, 10% of all enteric disease, and the actual PAF measured through epidemiological studies. If the PAF exceeded 10% then appropriate health and water quality targets could be set. However, different countries may wish to set such a level at a lower or higher level than this.

Setting a reference level of acceptable risk that is an absolute risk would be even more difficult, since what is acceptable in absolute terms will vary depending on the prevailing prevalence or incidence of disease and mortality related to that disease, and on social and economic conditions and technical capacity to implement interventions successfully.

6. Conclusions

The review of epidemiological studies of wastewater and excreta use in agriculture shows that there are significant risks of gastro-intestinal infections to consumers of crops, farm workers and their families and nearby populations exposed to untreated wastewater or excreta. Studies show that wastewater or excreta treatment prior to reuse can reduce these risks, and provide some indication of the extent of wastewater treatment needed to protect exposed populations against risks from helminth, bacterial and viral infections. However, there are methodological problems with many of the studies, and the results need to be interpreted with caution.

Analytical epidemiological studies provide better estimates of the magnitude of the risks from waste use in agriculture than descriptive studies. The main factors that affect the validity of the reported studies are selection bias, information bias due to misclassification of exposure or disease, lack of statistical testing of the association between exposure and disease, and lack of control for potential confounding factors. A sub-set of studies which dealt with these factors was used to provide the main estimates of the relative risk of infection related to particular exposures, estimates of threshold levels below which no excess infection in the exposed population could be expected, and measures of the proportion of disease in the study population attributable to exposure (and therefore potentially preventable through improvement in wastewater quality). Although these studies provide more data than was available in 1987 for the revision of WHO guidelines, there are still fewer studies available than would be desirable for the setting of international guidelines. Proposals on appropriate microbiological guidelines for wastewater and excreta reuse in agriculture are supported by the results of microbiological and risk assessment
studies (Table 15). However, local circumstances and concepts of acceptable risk should be taken into account when adoption of these guidelines are considered.

For unrestricted irrigation, there is evidence to suggest that there are increased risks of enteric infections (in a rural population in a developing country) when the water quality exceeds the WHO faecal coliform guideline of \( \leq 1000 \) FC/100ml by a factor of ten, so the guideline level needs to be below \( 10^4 \) FC/100ml. There is no evidence supporting any need to reduce the guideline level below \( 1000 \) FC/100ml in such circumstances. There is also no epidemiological evidence to indicate the threshold level when wastewater use is introduced to a naïve population. For protection against helminth infections, a nematode egg guideline of \( \leq 1 \) nematode egg per litre may be adequate where vegetables are grown commercially in hot and dry conditions and the time between harvest and consumption is prolonged. However, there is epidemiological evidence that treating the wastewater to the level of \( \leq 1 \) nematode egg per litre may not be sufficient where flood irrigation is used and field vegetables are eaten by the local population; a revised guideline of \( \leq 0.1 \) egg per litre may be necessary.

For restricted irrigation, it appears prudent to introduce a faecal coliform guideline to protect farm workers, their children, and nearby populations from enteric viral and bacterial infections. The appropriate guideline will depend on which irrigation method is used and who is exposed. If adult farm workers and nearby populations are exposed through spray/sprinkler irrigation, a guideline of \( \leq 10^3 \) FC per 100ml would be prudent (B1). The epidemiological evidence suggests that a reduced guideline of \( \leq 10^3 \) FC per 100ml would be safer where adult farm workers are engaged in flood or furrow irrigation or where children under 15 years are regularly exposed (through farm work or play) (B2 and B3). However, a faecal coliform guideline of \( \leq 10^3 \) FC/100ml could be adopted if effective health promotion measures could be taken to help adult farm workers and children improve hygiene measures post-wastewater contact. Regarding the risk of helminth infections, there is evidence to suggest that the nematode egg guideline of \( \leq 1 \) egg per litre is adequate for spray/sprinkler, flood or furrow irrigation if no children are exposed (B1), but a revised guideline of \( \leq 0.1 \) egg per litre would be safer if children are in contact with the wastewater through irrigation or play (B3). The level is set at around the limit of detection of nematode eggs in wastewater as transmission can occur at very low egg concentrations, and sampling methods are not very sensitive (so if any eggs are detected, transmission is probably occurring). However, if the proportion of the population who are exposed in this way is small, treatment to the level of \( \leq 1 \) nematode egg per litre could be combined with measures to restrict the contact of children with wastewater through play, if treatment to below the level of \( \leq 1 \) nematode egg per litre could not be provided. Where localised irrigation of a restricted range of crops occurs, treatment is required to allow the irrigation technology to function, and to protect the health of workers who have to clean the sprinkler nozzles.

Studies of the use of excreta or ‘nightsoil’ to fertilize vegetables indicate that effective treatment of excreta before use (for example, through use of chemical ovicide) can lead to reduced re-infection rates such that a large reduction in the prevalence of Ascaris and hookworm (and to a lesser extent, Trichuris) infections in exposed populations can be achieved. There is insufficient evidence of the effectiveness of traditional treatment or composting of faeces in reducing the prevalence of Ascaris and hookworm infection in exposed persons. No changes are proposed to guidelines for excreta use, other than those implied by changes to the guidelines for wastewater use in agriculture.
<table>
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<tr>
<td>A</td>
<td>Unrestricted irrigation</td>
<td>Workers, consumers, public</td>
<td>Any</td>
<td>0.1 - 1f</td>
<td>≤ 10³</td>
<td>Well designed series of waste stabilization ponds (WSP), sequential batch-fed wastewater storage and treatment reservoirs (WSTR) or equivalent treatment (e.g. conventional secondary treatment supplemented by either polishing ponds or filtration and disinfection)</td>
</tr>
<tr>
<td>A1</td>
<td>Vegetable and salad crops eaten uncooked, sports fields, public parks^e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Restricted irrigation</td>
<td>B1 Workers (but no children &lt;15 years), and nearby communities</td>
<td>(a) Spray/sprinkler</td>
<td>≤ 1</td>
<td>≤ 10³</td>
<td>Retention in WSP series inc. one maturation pond or in sequential WSTR or equivalent treatment (e.g. conventional secondary treatment supplemented by either polishing ponds or filtration)</td>
</tr>
<tr>
<td>B2</td>
<td>as B1</td>
<td>(b) Flood/furrow</td>
<td>≤ 10⁵ - ≤ 10⁶h</td>
<td></td>
<td>As for Category A or B1, as appropriate</td>
<td></td>
</tr>
<tr>
<td>B2 as B1</td>
<td>Cereal crops, industrial crops, fodder crops, pasture and trees^g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>Workers including children &lt;15 years, and nearby communities</td>
<td>(c) Any</td>
<td>0.1 - 1i</td>
<td>≤ 10³ - ≤ 10⁵h</td>
<td>As for Category A or B1, as appropriate</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Localised irrigation of crops in category B if exposure of workers and the public does not occur</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>None</td>
<td>Trickle, drip or bubbler</td>
<td></td>
<td>Not applicable</td>
<td>≤ 10³</td>
<td>Pretreatment as required by the irrigation technology, but not less than primary sedimentation.</td>
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a In specific cases, local epidemiological, sociocultural and environmental factors should be taken into account and the guidelines modified accordingly.
b *Ascaris* and *Trichuris* species and hookworms; the guideline is also intended to protect against risks from parasitic protozoa.
c During the irrigation season (if the wastewater is treated in WSP or WSTR which have been designed to achieve these egg numbers, then routine effluent quality monitoring is not required).
d During the irrigation season (faecal coliform counts should preferably be done weekly, but at least monthly).
e A more stringent guideline (≤ 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.
f If surface irrigation is used in areas where conditions are likely to favour the survival of helminth eggs, and field vegetables are eaten by the local population (i) this guideline should ≤0.1 egg per litre, or (ii) wastewater treatment should be supplemented with anthelmintic chemotherapy campaigns in areas of wastewater re-use.
g In the case of fruit trees, irrigation should cease two weeks before fruit is picked and no fruit should be picked off the ground. Spray/sprinkler irrigation should not be used.
h where flood/furrow irrigation is used or where children under 15 years are exposed, then a faecal coliform guideline of 10⁵ FC/100ml should be adopted or a guideline of 10⁷ FC/100ml could be adopted if effective health promotion measures can be taken to encourage farm workers and children to improve hygiene measures post wastewater contact.
i Where children under 15 years are exposed (i) this guideline should be ≤0.1 egg per litre or (ii) wastewater treatment should be supplemented with anthelmintic chemotherapy campaigns in children in areas of wastewater re-use.
7. References


Vegetables May Serve as One Route of Transmission. The Journal of Infectious Diseases 168, 222 – 6


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