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Studies of food hygiene and diarrhoeal disease

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Contamination of weaning foods and water with enteropathogenic micro-organisms has been recognised in the past, but its link with the development of diarrhoea by young children in developing countries is lacking. This may explain the unavailability of effective interventions to reduce the risk of diarrhoeal diseases from this contamination. The frequency of contamination of weaning foods with enteropathogens is high in developing countries, and is dependent on the food type, storage time and ambient temperature of storage, the method used, and the temperature reached on re-warming before re-feeding. Other considerations are the bacterial content of cooking and feeding utensils. Fruit and raw vegetables can become contaminated with enteropathogenic micro-organisms by sewage-containing irrigation water, by washing produce and fruits in contaminated water, and how they are processed at home. In most studies reviewed, the level of contamination is higher in weaning foods than in drinking water. Since there is a need to reach a critical level of contamination before illness can occur after the ingestion of an enteropathogen, it is postulated that weaning foods are probably more important than drinking water for transmission of diarrhoeal diseases in developing countries. Several potential interventions have been identified, which should be developed and tested in controlled trials in developing countries. These interventions are needed to reduce contamination of weaning foods in households from developing countries, while adequate facilities for the provision of clean water and sanitation to those communities are placed.

Keywords: Contamination; weaning food; diarrhoea; intervention; enteropathogen; clean water; sanitation; developing world; community.

Introduction

In recent years many papers have been published documenting how both weaning food and water given to children contain substantial amounts of bacteria (Barrell and Rowland 1979; Black *et al*. 1982, 1989; Molbak *et al*. 1989). However, the relationship between contaminated weaning foods and diarrhoeal diseases has not been clearly documented (Roberts 1990). Moreover, interventions to reduce bacterial contamination of food have not been fully developed in less developed countries.

Many people believe water to be the most significant route of transmission of agents causing diarrhoeal diseases. There are only rare instances where food has been implicated as the source of diarrhoea, mostly in diarrhoea outbreaks and practically all reported in developed countries. Very few, if any, disease outbreaks have been reported as caused by food in developing countries. It is not that food in developing countries is safe, rather that data are not collected and published regarding the incidence of disease due to food. The role of food as a risk factor for diarrhoeal diseases in developing countries is suspected when travellers from developed

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countries visit developing countries and suffer diarrhoea from eating contaminated food (Merson *et al*. 1976; Tjoa *et al*. 1977). Moreover, evidence reviewed in the literature clearly documents how food may play an important role in the epidemiology of diarrhoeal diseases in developing countries.

Epidemiological evidence of weaning foods as a cause of diarrhoea

In the early 1960s it was first recognised that at the point when children were introduced to weaning foods many began to suffer from frequent bouts of diarrhoea and to falter in their growth (Gordon *et al*. 1963). The timing of the peak incidence of diarrhoea coincided with ages when greater amounts of weaning foods were consumed. Breastfeeding was recognised as protective against diarrhoeal diseases, both by immune mechanisms as well as by delaying the introduction of contaminated weaning food, which was particularly beneficial in children aged under 6 months who are highly vulnerable (Brown *et al*. 1989; de Zoysa *et al*. 1991). In the early 1980s, research groups tried to link contaminated weaning foods to diarrhoea produced in a child with particular enteropathogens, but there were conflicting results, as in two case studies carried out in Bangladesh and Gambia (Black *et al*. 1981, 1982; Lloyd-Evans *et al*. 1984).

Conflicting results were also obtained in longitudinal studies. Again, initial studies found an association between the amount of food contaminated with faecal coliforms and the incidence of diarrhoea due to enterotoxigenic *Escherichia coli* (Black *et al*. 1982; Bukenya and Nwokolo 1990), but in later studies in the same country no association was found between the level of *E. coli* contamination of food or water and diarrhoea prevalence (Henry *et al*. 1990; Han *et al*. 1991). Perhaps the study methods used to check for foodborne pathogens and enteropathogens in food were insensitive, or the studies were poorly designed and under-powered.

In general, few people recognise that pathogens that cause diarrhoea come predominantly from food. Within foods, enteropathogens are mixed with the endogenous microflora of the food, and are not universally or uniformly distributed within the food. Thus, if the wrong spot is selected for culturing, or the microbiological method used does not eliminate other flora competing with the nutrients of the agar plate, the growth of the enteropathogen may be obscured, making identification difficult, if not impossible, in the culture media. New improved methods for food microbiology need to be found because those currently used are not sensitive enough or appropriate in identifying pathogens mixed with normal food flora.

Microbial content of raw foods

Studies have shown how food is an important transmitter of enteropathogens. Raw ingredients for preparing meals and uncooked foods left at home have been reported to contain a range of bacteria, viral or parasitic enteropathogens, in 30–80% of samples studied (Geldreich and Bordner 1971; Rodriguez-Rebollo 1974; Jiwa *et al*. 1981; Abdelnoor *et al*. 1983). There are between 10^3 and 10^8 micro-organisms per gramme, the number of which certainly mounts up when the weight of an average meal is considered. For example, in a salad, which does not contain any cooked ingredients, several millions of potentially pathogenic micro-organisms are ingested in a single serving.

In Santiago, Chile, research was carried out to ascertain why typhoid fever in the 1980s had a higher incidence in the highest socio-economic class than those in lower socio-economic groups. Results of studies showed that chronic carriers of *Salmonella typhi* could cause transmission of typhoid fever through sewage. On leaving the household sewage was channelled into the river

Mapocho to an area south of Santiago and used for irrigation of cultivated crops, including tomatoes and lettuce. The best produce sold on the market happened to be that grown closer to the market, which can be harvested when fully mature and be less affected by transportation. This better produce was sold in those markets in Santiago that served higher socio-economic groups, paying higher prices, and who also have the custom of consuming salads in large quantities.

To prove this hypothesis, an intact tomato was dipped into a solution containing *S. typhi* and left for 24 h at room temperature. On cutting into the tomato, the count was approximately one million *S. typhi* per gramme of tomato in the core. The bacteria penetrated the tomato, even through an intact surface (Samish and Etinger-Tulczynska 1963; Samish *et al.* 1963). When similar experiments were done with cucumbers, the highest concentration of pathogens was found close to the surface (Meneley and Stanghellini 1974), showing that the distribution of enteropathogens was not uniform and depended on the vegetable tested. As a result, even thorough washing of these vegetables with water or bactericidal solutions does not remove the bacteria because a large proportion can be found inside the vegetables, where water or bactericidal solutions cannot reach them. Those vegetables that grow close to the soil have the higher probability of being contaminated with bacteria, while fruit that grows high in trees are clean (Abdelnoor *et al.* 1983). Thus, there is a kind of contamination gradient at work, depending on the type of vegetable and its proximity to a contaminated soil or irrigation water.

How does food become contaminated? Normally, enteropathogens are not present in these foods, therefore contamination with fluids containing human faecal material seems the most obvious source. The idea that the purpose of a public health officer is fulfilled when sewage is piped out from the home is not sound. People have yet to realise that sewage and the pathogens it contains can return to the household indirectly through contaminated food products when untreated sewage is used for irrigation.

Sewage-contaminated water is a highly appreciated source of irrigation for agriculture in developing countries. It has more value than water alone because it contains fertilisers in the form of contaminated sewage, which is used for irrigation (Editorial 1990; Downs *et al.* 1999). In parts of Lima, Peru, for example, holes are made in sewage collectors to capture the sewage and distribute it in irrigation channels to farm fields. The crop yield is much-increased with healthy looking vegetables being sold for a high price in the best markets. In addition, things become worse when growers need to wash their harvest to remove the soil from the produce, but do so by washing the vegetables together in heavily contaminated sewage-containing water. Later, when the produce arrives at the market, it needs to be kept fresh in order to sell it. Again, heavily contaminated water is often used by people in the market stalls to keep the vegetables fresh-looking, further contaminating tomatoes, lettuce and other vegetables with bacteria and other pathogens (Geldreich and Bordner 1971). In contrast, there is less contamination of produce only for 'privileged' sources of crops that are irrigated with well water or potable water to produce a safe, 'organic' crop, which only few people can afford given its higher price. To complete the process of sanitation in any country, sewage disposal piped outside the home needs to be treated *before* it reaches the river or ocean.

Microbial content of weaning foods

Many studies have been carried out on weaning foods given to children at the point just before consumption. Faecal coliforms were found in a high proportion of the foods, but the counts

were not equally distributed, being found in 8–98% of samples at a level of contamination of between 10^3 and 10^8 bacteria per gramme dependent on the type of food tested (Barrell and Rowland 1979; Black *et al.* 1982, 1989; Molbak *et al.* 1989;). It has been shown consistently that foods that require to be cooked and eaten while still hot have a lower level of contamination than foods eaten raw, or when cool, or those consumed a long time after being cooked. Milk always contains a high number of faecal coliforms than many other foods (Barrell and Rowland 1980; Agarwal *et al.* 1982), and universally, the amount of bacteria found in food is much higher than in water.

One factor that explains how drinking water cannot have the same level of contamination as food, is turbidity. If a solution contains 10^6 bacteria per cubic centimetre it is very turbid as the bacteria are plainly visible, and therefore, it will not be consumed. On the other hand, if 10^6 bacteria are found per gramme of food which is not visible, nor does it smell, it is eaten. The bacteria seem to be part of the taste of the food. Some foods, like those that are fermented or acidic are more resistant to bacterial contamination. It is these kinds of food that are being promoted because of this property (Mensah *et al.* 1990).

Storage affects contamination of weaning foods

The level of contamination of food is dependent on the time between preparation and consumption. If cooked food is eaten within 1 h of being prepared it should be safe most of the time, but very seldom do mothers in developing countries adhere to this timeline (Black *et al.* 1982, 1989). Food cooked earlier in the day is re-heated at night, so most foods stored 4–6 h after preparation are heavily contaminated with bacteria (Rowland *et al.* 1978; Barrell and Rowland 1979; Black *et al.* 1989). Bacteria can replicate very rapidly, increasing from 10^3 to 10^6 bacteria per gramme of food within 3 h, the level of which usually reaches a plateau around 8 h after storage in ambient temperatures. In addition, if food is left uncovered then higher levels of contamination occur than covered food, partly because of the role of flies in transferring contaminants to food.

Temperature affects contamination of weaning foods

Temperature is important when considering level of contamination. Food becomes more heavily contaminated in the summer than in the winter, and it is important to recognise that temperatures between 20 and 40°C are optimal for the growth of bacteria in food, while those temperatures below 6 or higher than 60°C are safer (Table 1) (Motarjemi and van Schothorst 1999). Refrigeration, in particular, is important in reducing the risk of contamination of stored food, as was discovered in the US population with industrialisation, when the number of people suffering with diarrhoea was reduced probably because of the fact that more households were able to afford refrigerators.

Problems arise when use of available refrigerators to store weaning foods compete with business use to store items, such as beer and other refreshments, as was observed in Peru. Thus, people need to be educated in making good use of refrigerators to store weaning foods and other foods that can be affected by high levels of contamination.

Cooking practice affects contamination of weaning foods

The cooking practice of most women in developing countries involves cooking once per day, around midday, which means that re-heating is usually employed for the evening meal, but the temperature used is very much below 60°C. As a result, the large number of bacteria that have grown since the time it was first cooked are not killed in sufficient quantities when the food is re-

Table 1. Minimum optimal and maximal temperature for the growth of pathogenic micro-organisms in food and water. Adapted from Motarjemi and van Scothorst (1999)

	Temperature ($^\circ$C)		
Micro-organism	*Minimum*	*Optimal*	*Maximum*
Salmonella	5	35–37	47
Campylobacter	30	42	47
Escherichia coli	10	37	48
Staphylococcus aureus	6.5	37–40	48
Clostridium botulinum	3–10	–	50
Bacillus cereus	4	30–35	43
Penicillium verrucosum	0	20	31
Aspergillus ochraceus	8	28	37
Aspergillus flavus	10 (12 for tox)	32 (25 for tox)	42 (37 for tox)
Fusarium moniliforme	3	25	37

heated in the evening. Moreover, cooking again or re-heating food to a high temperature may not be economically feasible (Gilman and Skillicorn 1985).

Hygiene practices are important, particularly in lower socio-economic households where there is a greater risk of contamination. Handling food increases the risk of contamination, which is easy to understand why when it was shown that 14–79% of mothers' hands in developing countries were contaminated with faecal coliforms (Black *et al*. 1989; Vadivelu *et al*. 1989; Imong *et al*. 1995).

Utensils affect contamination of weaning foods

Utensils, even the so-called 'clean' utensils (empty bowls, cups, spoons, etc.) provided by mothers for culturing, were found to have very high counts of bacteria, including enteric coliforms, in Peru and Gambia (Rowland *et al*. 1978; Barrell and Rowland 1980; Black *et al*. 1989).

A study in Peru showed how milk can become contaminated (Black *et al*. 1989). Evaporated milk is sterile when the container is opened. However, because the can opener used to open the milk tin is usually not cleaned, it acts as a reservoir for the bacteria, which are then introduced into the milk at the time of opening. When the milk was left at room temperature and cultured 2–4 h later, the milk had a concentration of 10^6 coliforms per cubic centimetre. The mother may boil the water and even boil the baby bottle to sterilise it, but they never boil the evaporated milk, which may have been exposed to the environment for several hours. In essence, a pure culture is being added to clean water providing the infant with milk containing a high concentration of bacteria.

Baby bottles affect contamination of weaning foods

Boiling water is required to prepare tea, which means that it should be sterile at the time of preparation. In a study in Peru, when tea was given to children after a period of time, it remained uncontaminated if served in a cup, but 35% of the samples were found to be contaminated with faecal coliforms if the tea was offered in a baby bottle (Black *et al*. 1989). Even 'cleaned' baby bottles are always heavily contaminated as they are very hard to cleanse

properly. There are multiple types and numbers of micro-organisms that cause contamination here, particularly in the nipple, ensuring a high bacterial count when the bottle is next used. Mothers in less developed countries usually do not have access to a sufficient number of bottles at home and therefore frequently re-use those that they have. The bottles are often not dried properly and even when boiling of bottles is reported, methods used do not sterilise the bottle and nipple, thus providing a source of permanent contamination (Barrell and Rowland 1980; Elegbe *et al.* 1982; Cherian and Lawande 1985).

Microbial contamination of drinking water

In less-developed countries, waterborne diseases, such as cholera, typhoid fever, giardiasis, Norwalk agent, Cryptosporidium and others have been reviewed (Esrey *et al.* 1985). It has been shown that the increased number of coliforms in drinking water is associated with diarrhoea and poor growth in these countries. A study in the Philippines showed that at least 10^3 coliforms per cubic centimetre, not an insignificant amount, were required for there to be a risk of diarrhoea (Moe *et al.* 1991).

The issue of manipulation of the water in the home is also important. The unintentional introduction of hands into water reservoirs and buckets has lead to increased levels of contamination (Saran and Gaur 1981; Yeager *et al.* 1991). But even though contaminated water has been associated with diarrhoea risk, contaminated food has not been so clearly documented. It is postulated that food must be more important than water for diarrhoeal disease transmission, simply because there is a much greater amount of bacteria in contaminated food than water, where a higher ingestion is associated with higher risk, as discussed below.

Risk for diarrhoea

Studies in human volunteers have shown that between 10^5 and 10^8 bacteria per cubic centimetre were needed in order for diarrhoeal diseases to develop in susceptible individuals (Levine *et al.* 1981). The challenge dose to initiate a diarrhoeal bout could be reduced if food was ingested as it buffered the gastric acid, making the chances of getting diarrhoea with a lower amount of bacteria more likely. *Shigella* and some of the enterohaemorrhagic *E. coli* require a much smaller dose of bacteria to initiate disease (Table 2). Much controversy exists in India and Bangladesh as to whether the prevalence of hypochloridia (and *H. pyloric* infections) may be a risk factor for diarrhoea and cholera (Schiraldi *et al.* 1974; Nalin *et al.* 1978).

Potential interventions

Starting from the agricultural field, the level of microbiological contamination of irrigation water can be reduced by affordable sewage treatment systems, like oxidising ponds, where solar rays destroy pathogenic bacteria if sewage is allowed to be exposed for an appropriate time. Proper legislation could also be passed to control produce grown with contaminated water, but in most places, it is hard to implement. Another alternative is to train farmers to postpone harvests until a period of 15 days from the last irrigation, allowing the natural flora of food to displace the human contaminants. If the produce is harvested more than 15 days after being irrigated with sewage the normal flora will have had a sufficient period to take over and destroy pathogens, and the soil would have become dry and pathogen-free. This would ensure a safer crop than harvesting immediately after irrigation.

Table 2. Minimum infective dose for micro-organisms associated with foodborne diarrhoea outbreaks. Adapted from Motarjemi and van Scothorst (1999)

Micro-organism	Minimum infective dose
Enteropathogenic E. coli	10^6
Enterotoxigenic E. coli	10^6
Enteroinvasive E. coli	$10-100$
Enterohaemorrhagic E. coli	10
Shigella	$10-100$
Salmonella	10^6 ($10-1000$ with fatty foods)
Salmonella typhimurium	$10-100$
Campylobacter	500
Vibrio cholerae	10^6
Staphyloccocus aureus	10^6
Clostridium botulinum	10^4-10^5
Clostridium perfringens	10^4-10^5
Bacillus cereus	10^7-10^8

There is also a need to observe practices carried out both in the food market and in the home, and to promote the use of recently cooked weaning foods instead of those prepared much earlier. More interventions are also required to discourage the practice of providing children with re-heated food at night. One method used, as shown by a study in Ghana, was to wrap the food in aluminium foil and place it in an air-tight plastic insulated container exposed to sunlight to maintain a temperature of approximately 60°C inside until consumed in the evening. Handwashing in the kitchen before, during, and after food preparation, and while cooking, should be encouraged, as should the proper cleaning of utensils. Also, kitchen cloths and mops should be allowed to dry properly or be exposed to sunlight to destroy pathogens.

Safer weaning foods, as previously described, need to be promoted, including fermented products such as yoghurts, probiotics and prebiotics. Use of baby bottles for feeding should be avoided as long as possible in favour of breastfeeding. Also, the quantity and quality of water should be improved. This has already been shown to provide benefits through the employment of several methods. These include point-of-use disinfection of contaminated water, which studies have indicated to be protective by reducing the incidence of diarrhoea in Bolivia (Quick *et al.* 1999); solar disinfection, which reduced the incidence of diarrhoea by 34% in Maasai children (Conroy *et al.* 1996); and the provision of safe water containers with a narrow neck to prevent contamination from the introduction of hands and foreign bodies, and boiling of water.

Conclusion

In conclusion, contamination of weaning food and drinking water are important in the transmission of diarrhoeal diseases in less developed countries. Water has been identified as an area where effective interventions should be promoted to improve its quality and quantity. The role of contaminated weaning food is still unrecognised and more studies are needed to document its impact and develop appropriate interventions for application in developing countries. This is particularly significant as it has been postulated that food, not water, may be the most important route of transmission of diarrhoea in less developed countries.

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