The microbiological quality of seven large commercial private water supplies in the United Kingdom

D. Kay, J. Watkins, C. A. Francis, A. P. Wyn-Jones, C. M. Stapleton, L. Fewtrell, M. D. Wyer and D. Drury

ABSTRACT

Some 1% of the UK population derives their potable water from 140,000 private water supplies (PWSs) regulated by Local Authorities. The overwhelming majority of these are very small domestic supplies serving a single property or a small number of properties. Treatment for such supplies is rudimentary or non-existent and their microbiological quality has been shown to be poor in every published study to date. Private water supplies serving commercial enterprises such as hotels, restaurants, food production premises and factories are more frequently treated and subject to closer regulation in the United Kingdom. As a result, it has been assumed that these larger commercial supplies are less likely to experience elevated faecal indicator and pathogen concentrations at the consumer tap which have been observed at small domestic supplies.

This paper reports on intensive monitoring at seven commercial private water supplies (six of which were treated) spread throughout the UK serving hotels, holiday parks and food production enterprises. Daily sampling of 'potable' water, both at the consumer tap and using large volume filtration for *Giardia* and *Cryptosporidium* spp. was conducted over two six week periods in the spring and autumn of 2000. This allowed the effects of short term episodic peaks in faecal indicator and pathogen concentration to be quantified. All the supplies experienced intermittent pathogen presence and only one, a chlorinated deep borehole supply, fully complied with UK water quality regulations during both periods of sampling.

Poor microbiological water quality typically followed periods of heavy rainfall. This suggests that the design and installation of such systems should be undertaken only after the likely range of raw water quality has been characterised, which requires a thorough understanding of the effects of flow and seasonality on raw water quality. There is no reason to suspect that the monitored sites are uncharacteristic of other commercial supplies and the results reinforce public health concerns related to domestic supplies. Furthermore, the pattern of contamination is highly episodic, commonly lasting only a few days. Thus, the relatively infrequent regulatory monitoring of such supplies would be unlikely to identify the poor water quality episodes and does not provide the data necessary for public health protection.

Although some statistical relationship was found between faecal indicator organisms and the presence of pathogens, the use of FIOs in assessments of regulatory compliance may not always provide a reliable measure of public health risk, i.e. indicator absence does not preclude pathogen presence. The results of this study suggest that a risk assessment system similar to the WHO 'Water Safety Planning' approach might offer a more appropriate regulatory paradigm for private water supplies.

Key words | faecal indicators, large private water supplies, pathogen, rainfall, waterborne disease, water quality

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INTRODUCTION

The 140,000 UK private water supplies (PWS) (Reid *et al.* 2003) are regulated by the Private Water Supply Regulations (1991) (Anon 1991). These require Local Authorities to sample and analyse water with the sampling frequency determined by the type of water supply. In the case of domestic supplies (Category 1) sampling frequency is based on the population served. For supplies used for commercial purposes (Category 2), for example hotels and food preparation, sampling frequency is based on the volume supplied. A series of studies in Europe and North America have demonstrated consistent and wide scale failure of many small, principally rural, domestic private water supplies against microbiological, chemical and radiological criteria (Conway 1981; Mathys & Soddemann 1988; Pollock 1992; Whitten 1992; Kross *et al.* 1993; Petrie *et al.* 1994; Constantine *et al.* 1995; Humphrey & Cruikshank 1995; Clapham & Horan 1996; Duke *et al.* 1996; Fewtrell & Kay 1996; Clapham 1997; Shepherd & Wyn-Jones 1997; Fewtrell *et al.* 1998; Chalmers *et al.* 2000; Dawson & Sartory 2000; Harrison *et al.* 2000; Rutter *et al.* 2000; Licence *et al.* 2001; Thompson 2001; Levin *et al.* 2002; Thompson 2003).

The public health implications and resultant disease burden are less well documented and the Infectious Intestinal Disease study in the UK (Anon 2000*c*) did not identify private water supplies as a major cause of gastrointestinal illness, although precise surveillance and subsequent recording of self-limiting gastrointestinal illness is notoriously difficult (Georgiou & Langford 2003). Case control studies seeking to examine any health differences between individuals consuming water from private supplies and treated public supplies have also failed to confirm a poorer health status in the former group (Meara 1989).

However, a review undertaken in 1987 suggested that in the period 1937 to 1986 thirteen disease outbreaks involving 1,900 cases could be attributed to private water supplies (Galbraith *et al.* 1987). More recently, seven cryptosporidiosis outbreaks were attributed to the consumption of water from private supplies (UKWIR 1998). The most recent and comprehensive assessment, led by staff of the UK Public Health Laboratory Service (PHLS) (now the UK Health Protection Agency (HPA)), concluded that, in the UK, 57% of drinking water related disease outbreaks had been associated with the consumption of water from private supplies (Said *et al.* 2003). *Campylobacter* was the principal aetiological agent in 52% of these outbreaks and commercial (Category 2) supplies accounted for 88% of the reported illness. The authors suggest that outbreaks related to water from private supplies are characterised by: (i) transient populations, (ii) inadequate or ineffective treatment, (iii) animal presence in the area around the supply and (iv) rainfall events preceding consumption of polluted water. Similar factors have been associated with a major *E. coli* O157 outbreak in Ontario affecting 2,300 people and causing seven deaths, which serves to outline the potential for serious harm from polluted potable water even within the most affluent and technologically advanced nations (Kondro 2000; Rose *et al.* 2000; Spurgeon 2000; Anon 2000*b*; Curriero *et al.* 2001). The UK PHLS-HPA study concluded that "*the number of cases and outbreaks associated with PWS and therefore the public health problem is probably underestimated. The commercial use of PWS potentially exposes larger populations to the risk of illness from contaminated water from PWS. Identification of risk factors, proper protection of water sources, and adequate treatment and maintenance are essential to protect these populations*" (Said *et al.* 2003).

This investigation focussed on commercial category 2 supplies which had up until the time of the study received little research attention. The supplies were anonoymised to maintain confidentiality.

METHODS AND MATERIALS

Site descriptions

Seven private water supply sites were selected across the UK; two in each of England, Scotland, and Wales and one in Northern Ireland (Table 1). The sites included holiday camps, a hotel, a hospital, a farm and a small rural village with a resident population of approximately 500. Site 1 was a chlorinated borehole source; site 2 a stream source with filtration and ultra-violet (UV) light disinfection; site 3 was a shallow well with filtration and UV disinfection; site 4 was

Table 1 | Details of private water supplies included within this study

Site	Source	Treatment	Virus samples
1	Borehole	Chlorination	No
2	Stream	Filtration & UV disinfection	No
3	Well	Filtration & UV disinfection	No
4	Reservoir	Filtration & chlorination	No
5	Springs fed stream	None	14 samples
6	Borehole	Filtration & ozonation	No
7	Resurgent underground stream	Filtration & chlorination*	25 samples†

*Treatment installed between spring and autumn sampling phases.
†20 untreated and 5 treated samples tested.

an open reservoir fed mainly by underground springs and some surface water input with filtration and chlorination; site 5 was an untreated spring-fed surface water source; site 6 was a borehole with filtration and ozonation; and site 7 was a resurgent underground stream from a karst groundwater system. This latter site had a filtration and chlorination treatment plant installed between the two sampling periods in the study.

Sampling techniques

The sites were sampled at the consumer tap at approximately 08:00 and 10:00 GMT daily for a period of six weeks in the spring (May and June) and six weeks in the autumn (end of September to mid-November) of 2000. Samples were analysed for coliforms, *Escherichia coli*, enterococci, presumptive *Clostridium perfringens* and *Campylobacter*. Additional samples were taken for *E. coli* O157:H7 if other water quality parameters (e.g. turbidity) suggested the potential for its presence. Samples were taken in sterile plastic bacteriological bottles containing sodium thiosulphate, transported to laboratories in cool boxes with ice packs and analysed within six hours of collection. Five laboratories participated in the analysis of the samples. Samples were also taken for virological analysis from sites 5 and 7.

Samples were acquired on a daily basis for *Cryptosporidium* and *Giardia* analyses using the UK regulatory *Cryptosporidium* monitoring cabinets and Genera Filta-Max™ filters and filter housings. The assembled housings were pressure tested to 5 bar for 15 min before use to ensure that they were leak-proof. Samples were transported overnight to the CREH *Analytical* laboratory in Leeds, UK for analysis on the next working day or stored by the sampling personnel at 4°C over weekends before being transported.

Samples for virological analysis were collected only during the second of the two periods of fieldwork i.e. autumn 2000 which included periods of heavy rainfall that resulted in widespread flooding throughout the United Kingdom. Thirty-nine samples were taken, of which 14 (20 litres, all untreated) were from site 5 and 25 (50 litres, 20 untreated and five treated) were from site 7. Samples were collected in sterile 10 litre bottles containing sodium thiosulphate.

Laboratory analysis

Coliforms and *E. coli* were enumerated on membrane lactose glucuronide agar (Anon 2000*c*, 2002*c*, *d*). Isolates were confirmed by the oxidase test, fermentation of lactose on MacConkey agar and indole production on 1% tryptone nutrient agar using Kovacs' reagent (Anon 2002*d*).

Enterococci were isolated by membrane filtration on Slanetz and Bartley agar, with incubation for 4 hours at 37°C and 44 hours at 44°C followed by confirmation of aesculin hydrolysis using kanamycin aesculin azide agar (Anon 2002*c*).

Presumptive *Clostridium perfringens* were enumerated on tryptone sulphite cycloserine agar, incubating at 44°C for 24 hours (Anon 2002*b*).

Campylobacter were isolated by enrichment in brain heart infusion broth containing Butzler antibiotic (Anon 2002*a*). Enrichment broths were subcultured to columbia agar base containing Preston antibiotics (Anon 2002*a*). Suspect colonies were subcultured and confirmed by Gram

stain. Isolates were typed by the Central Public Health Laboratory, Colindale, London.

Escherichia coli O157:H7 were isolated by membrane filtration (Anon 2000*a*). Typical colonies were confirmed using latex agglutination (Oxoid DR0620M). Isolates were confirmed at Sheffield (UK) Public Health Laboratory.

Cryptosporidium and *Giardia* samples were processed by the standard operating protocol outlined in Anon (1999). Samples were cleaned using immunomagnetic separation (Dynal GC Combo Kit, Dynal, UK). Slides were stained using two specific monoclonal antibodies (CelLabs, TCS Biosciences Limited, Buckingham, UK) and 4′, 6-diamidino-2-phenylindole (DAPI) (Sigma Aldrich, UK). Positive slides were examined using a 100x oil immersion to identify characteristic features. Presumptive *Cryptosporidium* oocysts were confirmed by appropriate size and the presence of sporozoite nuclei and presumptive *Giardia* cysts by appropriate size and the presence of nuclear material demonstrated by DAPI staining. Bodies in which no characteristic features were discernable with DAPI were examined by differential interference contrast and counted as empty parasites where morphology and morphometry were typical. Spearman's Rank Correlation Coefficient (r_s) was used to assess the relationships between the protozoan parasite and bacteriological parameters.

Rainfall data

Daily rainfall (mm) data were obtained for the UK Meteorological Office raingauge nearest to each site and for which a complete record of rainfall was available for both phases of the study. All gauges were within 15 km of the sample sites. The daily rainfall data comprised the total rainfall for the 24 hours prior to 09:00 GMT on any given date. The statistical significance of relationships between rainfall for the 24 hours prior to the collection of the sample (coincidentally at approximately 09:00 GMT) was correlated with the bacteriological and protozoan data using Spearman's Rank Correlation Coefficient (r_s). Correlation analyses were carried out for each phase (i.e. spring – phase 1 and autumn - phase 2) and for the combined data set, with the exception of site 7 where installation of treatment between the two phases of study precluded the combination of the data from each phase. Differences in median concentrations before and after installation of treatment at site 7 were analysed using the Mann-Whitney (U) test. All statistical test results were assessed at $\alpha = 0.05$ (i.e. 95% confidence level or 5% significance level).

RESULTS

A total of 1178 samples were analysed, mostly for bacteria and protozoan parasites. The results are summarised in Tables 2 to 6. Each site was sampled on approximately 40 consecutive days over each of the two phases. Figures 1 to 6 show the daily faecal indicator and protozoan parasite concentrations in the six supplies which were positive for these organisms.

Table 2 | Percentage of bacteriological failures for each site

Site	Sampling Phase*	Coliforms (%)	*E. coli* (%)	Enterococci (%)	Clostridia (%)
1	Phase 1	0	0	0	0
	Phase 2	0	0	0	0
2	Phase 1	65.0	65.0	25.5	51.0
	Phase 2	47.0	47.0	14.3	16.6
3	Phase 1	0	0	0	0
	Phase 2	0	0	0	2.4
4	Phase 1	4.8	0	0	2.4
	Phase 2	0	0	7.0	76.6
5	Phase 1	90.6	90.6	51.2	9.3
	Phase 2	100	100	69.7	14.0
6	Phase 1	2.4	0	0	0
	Phase 2	92.8	64.3	38.0	54.8
7	Phase 1	83.3	71.4	47.6	90.5
	Phase 2	27.3	26.1	6.8	61.4

*Sampling Phase: Phase 1: May and June 2000; Phase 2: October to mid-November 2000.

Table 3 | Maximum faecal indicator organism counts obtained from each site

Site	Sampling Phase*	Coliforms cfu 100 ml^{-1}	*E. coli* cfu 100 ml^{-1}	Enterococci cfu 100 ml^{-1}	Clostridia cfu 100 ml^{-1}
1	Phase 1	0	0	0	0
	Phase 2	0	0	0	0
2	Phase 1	82	82	3	11
	Phase 2	90	90	38	7
3	Phase 1	0	0	0	0
	Phase 2	0	0	0	1
4	Phase 1	10	0	0	1
	Phase 2	0	0	2	>100
5	Phase 1	30,000	15,000	2,400	3
	Phase 2	1,600	1,600	114	6
6	Phase 1	4	0	0	0
	Phase 2	>3,000	>3,000	>300	>300
7	Phase 1	6,300	6,300	470	140
	Phase 2	175	71	9	81

*Sampling Phase: Phase 1: May and June 2000; Phase 2: October to mid-November 2000.

Bacteriological results

The current standards for coliforms, *E. coli*, and enterococci in drinking water are zero in 100 ml of sampled water whilst for *Clostridium perfringens* the standard is <1 cfu 20 ml^{-1}. Only one site (site 1) met these standards throughout the study whilst site 3 met all but the *Clostridium perfringens* standard. Of the other five sites, the failure rate for coliforms and *E. coli* was between 2.4% and 100% (Table 2). The highest concentrations observed were at site 5: 30,000 coliform 100 ml^{-1} and 15,000 *E. coli* 100 ml^{-1} (Table 3). Two sites had no failures for enterococci (again, sites 1 and 3). The failure rate for the other five sites was between 6.8% and 69.7% with the highest count being 2,400 enterococci 100 ml^{-1} (site 5). Of the six sites positive for presumptive *Clostridium perfringens*, the failure rate was between 2.4% and 90.2% with the maximum concentration in excess of 300 cfu 100 ml^{-1}. At site 5, presumptive *Clostridium perfringens* was a very poor indicator of water quality in terms of numbers detected, both during phase 1 and phase 2 (Figure 4). At sites 4 and 7 presumptive *Clostridium perfringens* appeared to be a better indicator of faecal contamination in terms of numbers detected, particularly during phase 2 (Figure 3 and Figure 6).

Table 4 | *Campylobacter* types identified during the study

Site No.	Species	Serotype	Phage type	R-type
Site 5	*Camp. jejuni*	Untypable	73	Sensitive
Site 5	*Camp. jejuni*	HS8	Untypable	C‡
Site 5	*Camp. jejuni*	HS8	RDNC*	Sensitive
Site 5	*Camp. jejuni*	HS13	73	No data
Site 5	*Camp. jejuni*	HS13	73	No data
Site 7	*Camp. coli*	Untypable	Untypable	NxCp†
Site 7	*Camp. lari*	HS19	Untypable	Sensitive
Site 7	*Camp. jejuni*	Untypable	RDNC[a]	Sensitive
Site 7	*Camp. lari*	Untypable	1	NxCp†
Site 7	*Camp. lari*	HS19	Untypable	Sensitive
Site 7	*Camp. lari*	HS19	Untypable	Sensitive
Site 7	*A. butzleri*	Untypable	No data	No data

*Reacted with the typing phages but did not conform to a designated type.
†Resistant to naladixic acid and ciprofloxacin.
‡Resistant to chloramphenicol.

Campylobacter spp. were isolated from site 5 on five occasions and from site 7 on six occasions. *Arcobacter butzleri* (previously *Campylobacter butzleri*) was isolated from site 7 on one occasion. The isolates, typed by the Central Public Health Laboratory, Colindale, London, are given in Table 4. *Escherichia coli* O157:H7 was isolated from site 7 on one occasion and this isolate was subsequently shown to possess VT2 and a*eae*A genes but not VT1 using DNA probes (Chapman 2000).

Protozoan parasites and viruses

Cryptosporidium was detected at all the sites. In Phase one the minimum failure rate was 2.4% (one sample in phase 2 at site 4,

Table 5 | Percentage positive and maximum counts obtained for *Cryptosporidium* and *Giardia* at each site

		Positive (%)		Counts 1,000 l^{-1}	
Site	**Sampling Phase***	***Cryptosporidium***	***Giardia***	***Cryptosporidium***	***Giardia***
1	Phase 1	2.6	0	1	0
	Phase 2	2.6	2.6	1	1
2	Phase 1	75.0	60.0	32	17
	Phase 2	50.0	42.5	36	167
3	Phase 1	2.7	2.7	1	1
	Phase 2	56.6	50.0	40	83
4	Phase 1	0	0	0	0
	Phase 2	2.4	12.1	4	24
5	Phase 1	33.3	15.4	84	102
	Phase 2	56.0	65.9	9	743
6	Phase 1	0	0	0	0
	Phase 2	10.0	29.0	34	44
7	Phase 1	52.2	57.7	2,848	3,446
	Phase 2	91.0	84.4	71	2,171

*Sampling Phase: Phase 1: May and June 2000; Phase 2: October to mid-November 2000.

$n = 41$) and the maximum failure rate was 91% (site 7, phase 2). The minimum count obtained was 1 in 1,000 litres of water and the maximum was 2,848 (Table 5). *Giardia* was also detected at all sites. The minimum failure rate was 2.6% (one sample in phase 1 at site 1, $n = 38$) and the maximum was 84.4% (site 7 phase 2). The minimum count obtained was 1 in 1,000 litres and the maximum was 3,446 in 1,000 litres.

Viruses were detected in water samples from both sites sampled for viruses (sites 5 and 7; see Table 6). Cytopathic effects (c.p.e.) characteristic of virus infection were seen in 26 of the 39 samples tested by BGM liquid culture (Table 6). However, the number of cultures showing plaques in the monolayer plaque assay was much lower, only two samples showed plaques characteristic of enterovirus infection, and even here the count was low, one plaque forming unit (pfu) being found in one 20 litre sample and two pfu in another. Cell suspensions were prepared from liquid cultures showing cytopathic effects and tested for polioviruses and

Table 6 | Viruses detection in samples from sites 5 and 7

		No. of positive samples			
		Cell culture		RT-PCR	
Site	**No. of samples**	**Liquid culture***	**Plaque assay†**	**Entero**	**Noro**
5	14	10	0	1	0
7	25	16	2	1	0

*Assessed by cytopathic effect in BGM cell culture.
†Two cultures showed plaques; one showed 1 plaque, the other showed 2 plaques.

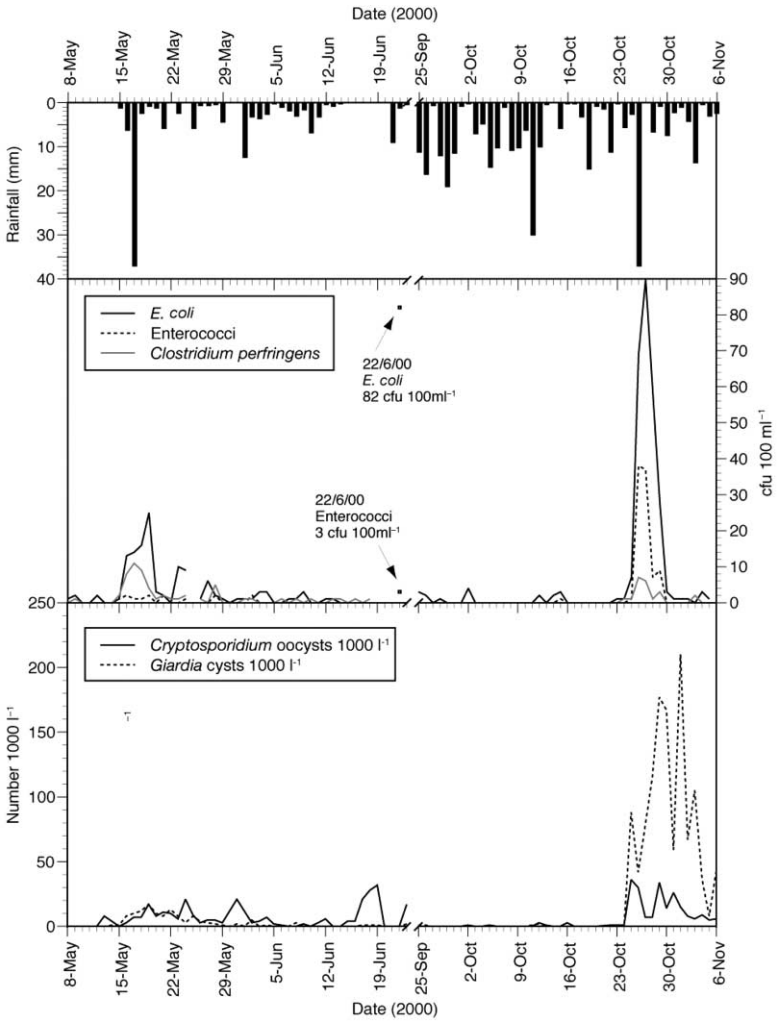

Figure 1 | Rainfall (mm), bacterial faecal indicator organism (cfu 100 ml^{-1}) and protozoan parasite concentrations (cysts/oocysts 1000 l^{-1}) at site 2.

Coxsackieviruses B by immunofluorescence (IF), but none confirmed positive.

Concentrates from two samples gave a positive c.p.e. in liquid culture and one initiated plaques in the plaque assay Thus the presence of enteroviruses suggested by the initial c.p.e. in liquid culture was confirmed in one sample by plaque assay and in both samples by enterovirus-specific RT-PCR, and this serves to illustrate the importance of confirmatory tests. Although both liquid culture-positive concentrates were confirmed by enterovirus-specific RT-PCR only one produced plaques; this is not unusual, echoviruses for example plaque only infrequently in BGM cell culture yet are found in environmental samples quite often. Concentrates were also tested by RT-PCR for the presence of noroviruses but none was detected.

Ten out of 14 samples from site 5 were positive in liquid culture, of which one was confirmed as enterovirus by RT-PCR. In site 7, 16 out of 25 samples were positive in liquid culture, again one being confirmed by RT-PCR. Two produced plaques in the monolayer assay.

Impact of rainfall

Sufficient positive bacteriological and protozoan results exist for comparison with rainfall during both phases of the

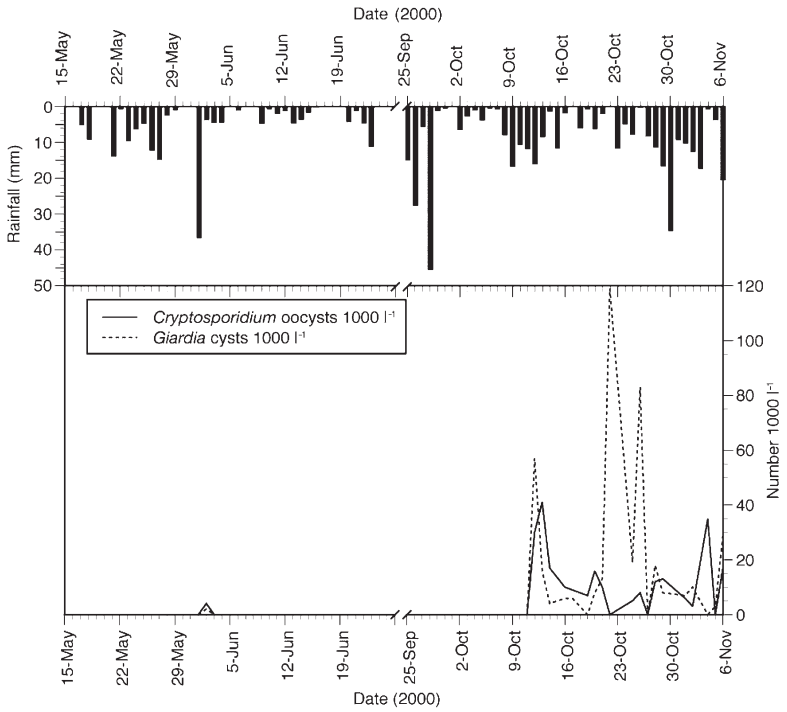

Figure 2 | Rainfall (mm) and protozoan parasite concentrations (cysts/oocysts 1000 l^{-}) at site 3. Note that there were no bacterial faecal indicator organisms in all samples.

study for sites 2, 5 and 7. Limited comparisons for phase 2 only were undertaken for *Cryptosporidium* and *Giardia* at site 3, for clostridia and protozoan parasites at site 4, and all bacterial indicators and protozoan parasites at site 6. The results of this analysis are shown in Table 7.

At site 2, the faecal indicator organisms (i.e. coliforms, *E. coli*, enterococci and *Clostridium perfringens*) were significantly correlated ($p \leq 0.003$) with rainfall during Phase 1 only (Table 7). However, inspection of the time-series plots for both phases (Figure 1) suggests that there may be a threshold-effect with coliforms, *E. coli*, *Clostridium perfringens*, *Cryptosporidium* and *Giardia* increasing after the high rainfall (37.0 mm) on 17/5/00, and again after 37.0 mm rainfall on 27/10/00 (enterococci also increased on this occasion). This hypothesised threshold would clearly be catchment specific and vary with antecedent rainfall. At site 5 all faecal indicator organisms and protozoan parasite concentrations were significantly correlated ($p \leq 0.005$) for the combined data from both periods, for coliforms, *E. coli*, enterococci and *Cryptosporidium* during phase 1 and all bacteriological parameters during phase 2 (Table 7). Again, the highest concentrations during each phase were associated with the highest rainfall totals on 17/5/00 (phase 1, 27.3 mm) and 25/10/00 (phase 2, 41.2 mm) (Figure 4). All faecal indicator organism concentrations were significantly correlated with rainfall ($p \leq 0.036$) during both phases at site 7, whilst *Cryptosporidium* and *Giardia* were also correlated with rainfall during Phase 2 (Table 7). As with sites 2 and 5, the highest concentrations of all parameters tested during phase 1 and protozoan parasites during phase 2 coincide with periods of high rainfall (Figure 6).

No statistically significant relationships with rainfall during phase 2 were evident for the protozoan parasites at sites 3 and 4 (also *Clostridium perfringens* at this site) (Table 7). However, in the case of site 4, elevated concentrations do coincide with higher rainfall on occasion (Figure 3). At site 6, all FIOs and *Giardia* were significantly correlated with rainfall during phase 2 (Table 7).

DISCUSSION

Only site 1 (a chlorinated deep borehole supply) had no bacteriological failures during both phases of sampling

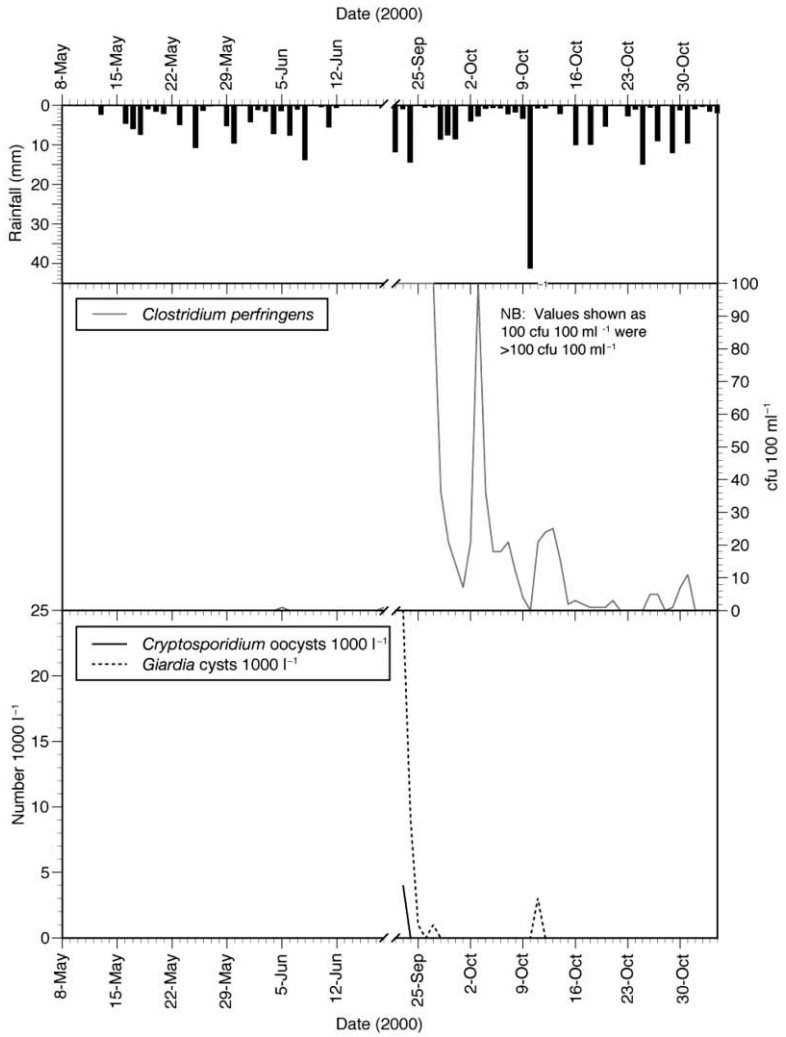

Figure 3 | Rainfall (mm), Clostridia (cfu 100 ml^{-1}) and protozoan parasite concentrations (cysts/oocysts 1000 l^{-1}) at site 4.

although one *Cryptosporidium* oocyst was detected in phase 2. Site 2 had a high percentage failure of indicator bacteria during both phases 1 and 2 which indicated the inadequacy of the filtration and UV treatment of this surface stream supply, particularly after rainfall (Figure 1). At site 2, the poor compliance against the faecal indicators was reflected in the consistently high presence of the protozoan pathogens and statistically significant correlations ($p \leq 0.002$) between the bacteriological parameters and both *Cryptosporidium* and *Giardia* (Table 8).

More surprisingly, at site 3, a well supply with filtration and UV disinfection, no coliform or enterococci failures were observed but over 50% of samples in the autumn survey contained *Cryptosporidium* and/or *Giardia* (Figure 2). Here, one sample was also positive for *Clostridium perfringens* but none of the regulatory 'indicator' species produced a reliable measure of risk for this supply. Site 4 exhibited a low level of coliform and enterococci failures in both sampling periods but surprisingly high levels of clostridia in phase 2. Protozoan parasites were present in one and five samples tested during the autumn period for *Cyptosporidium* and *Giardia* respectively (Figure 3). *Giardia* was significantly correlated with clostridia during phase 2 ($p = 0.001$) (Table 8).

Site 5 was the only untreated surface water examined. Unsurprisingly, this site had high rates of faecal indicator isolation and high rates of protozoan and bacterial (*Campylobacter*) pathogen presence in both the sampling

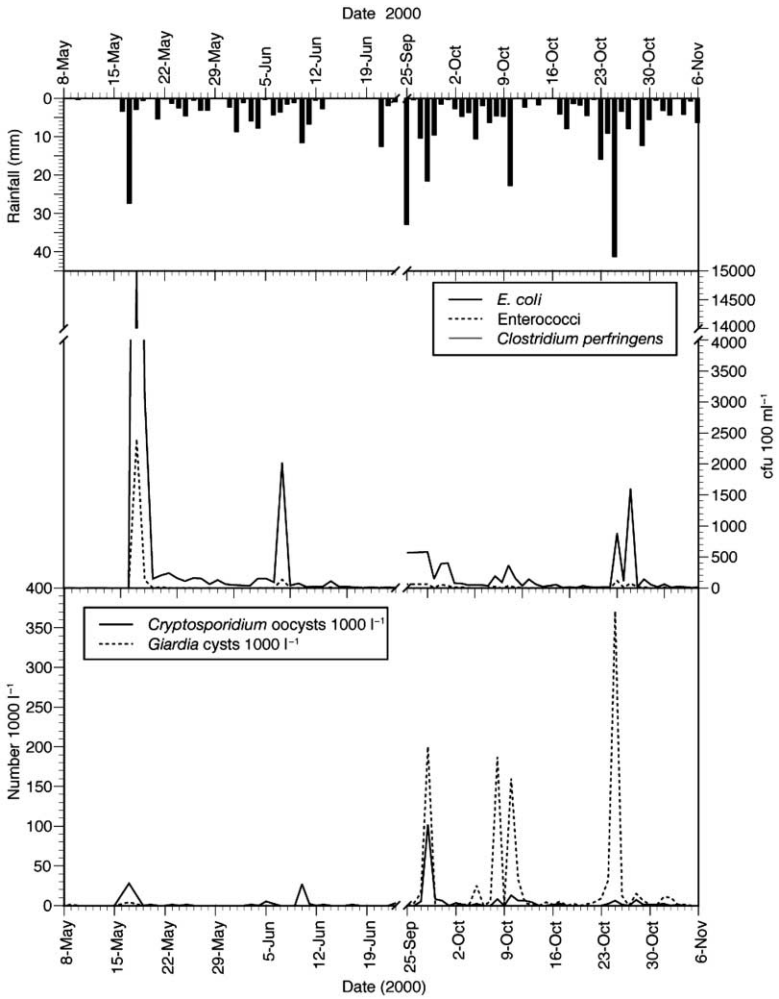

Figure 4 | Rainfall (mm), bacterial faecal indicator organism (cfu 100 ml^{-1}) and protozoan parasite concentrations (cysts/oocysts 1000 l^{-1}) at site 5.

periods (Figure 4). Here, coliforms, *E. coli* and enterococci were significantly correlated ($p \leq 0.01$) with *Cryptosporidium* whilst coliforms, enterococci and clostridia were significantly correlated ($p \leq 0.046$) with *Giardia* (Table 8). The ozone treated borehole supply serving site 6 produced only one faecal indicator failure in the spring sampling. However, this site exhibited a significant deterioration in the autumn phase when 10% and 29% of samples contained *Cryptosporidium* and *Giardia* respectively (Figure 5), but in low to moderate concentrations compared to the more polluted sites (i.e. sites 2, 5 and 7). Both *Cryptosporidium* and *Giardia* were significantly correlated ($p \leq 0.042$) with the bacterial parameters during phase 2 (Table 8).

Site 7 offered an interesting opportunity to examine the effects of a new treatment system employing filtration and chlorination which was installed between the spring and autumn sampling phases. This clearly reduced concentrations and the percentage of faecal indicator failures but it appeared to have had no discernable impact on the percentage of samples positive for the protozoan parasites, or indeed, the magnitude of *Giardia* concentrations (Figure 6). This site had the highest concentration of *Giardia* observed in both phases of sampling and the highest number of samples positive for *Campylobacter*. During phase 1, *Giardia* was significantly correlated ($p \leq 0.008$) with all bacteriological parameters although *Cryptosporidium* was significantly correlated ($p = 0.001$) only with *E. coli* (Table 8). All bacteriological parameters were significantly correlated ($p \leq 0.029$) with both *Cryptosporidium* and *Giardia* during phase 2 (Table 8)

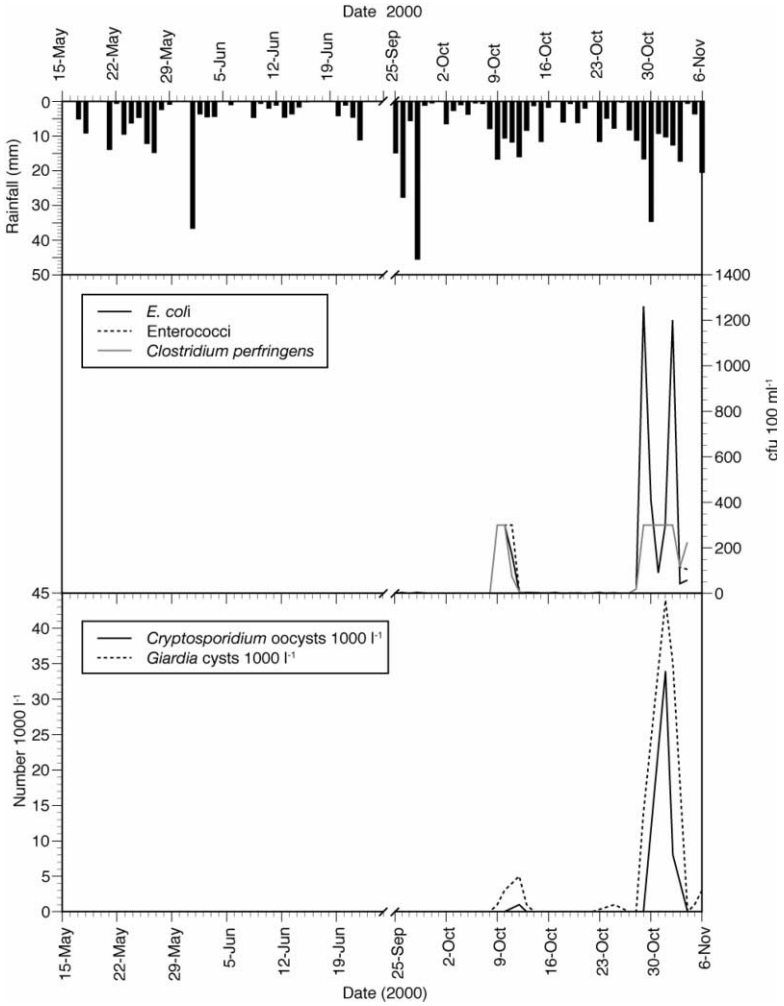

Figure 5 | Rainfall (mm), bacterial faecal indicator organism (cfu 100 ml^{-1}) and protozoan parasite concentrations (cysts/oocysts 1000 l^{-1}) at site 6.

despite their relatively low concentrations. The non-parametric Mann-Whitney (U) test indicated that the median concentrations of all bacterial indicators were significantly lower (all $p < 0.003$) during phase 2 after installation of the treatment system. However, similar tests for the protozoan parasites indicated that concentrations were significantly higher (both $p \leq 0.001$) during phase 2.

Sites 2, 5 and 7 were the supplies most consistently contaminated with viral pathogens and *Campylobacter* was isolated in phase 2 samples from sites 5 and 7. The borehole supplies tested during this study (sites 1 and 6) produced better quality water compared with other sources. The correlations with rainfall at the borehole supply of site 6 for phase 2 is perhaps surprising given that travel times between the surface and the groundwater are likely to be longer than the maximum period of 24 hours inherent in the rainfall data tested herein. This may also indicate by-pass flow possibly produced by insufficient protection of the bore hole or an ineffective casing.

Surface water supplies appeared to be more variable in quality and are likely to be more susceptible to the impacts of rainfall. Increased runoff resulting from rainfall causes saturated overland flow and stream level rise over bank areas accessed by grazing livestock, providing a pathway for faecal indicator and protozoan parasite delivery to surface water courses, whilst increased velocities within streams may re-entrain organisms present in bed sediments

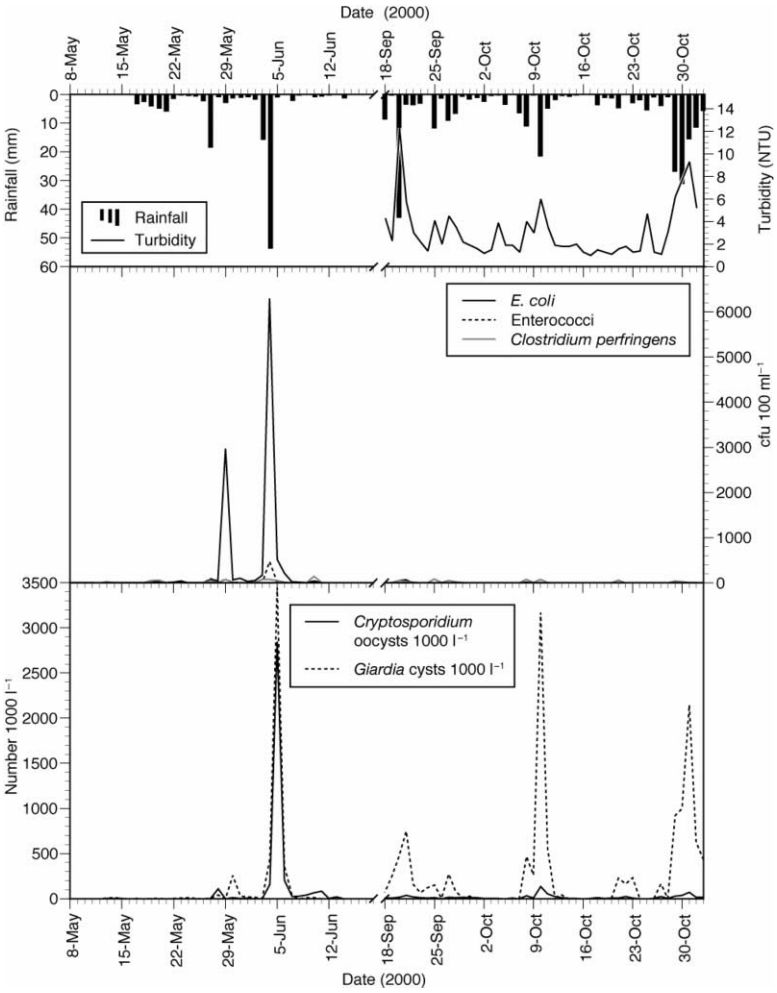

Figure 6 | Rainfall (mm), turbidity (NTU) bacterial faecal indicator organism (cfu 100 ml^{-1}) and protozoa concentrations (cysts/oocysts 1000 l^{-1}) at site 7.

(McDonald & Kay 1981; Jenkins *et al.* 1983; Wilkinson *et al.* 1995). Because of their hydrogeologic characteristics, karst aquifer systems such as that at site 7, are also susceptible to contamination from faecal indicator bacteria and pathogens, particularly by sediment attached organisms which become resuspended during periods of high flow induced by rainfall (Kay & McDonald 1983). Indeed, turbidity concentrations (NTU) at site 7 during the autumn period were generally greater during periods of higher rainfall (Figure 6).

The time series plots (Figures 1 to 6) also show that the bacterial indicator and protozoan parasite response to rainfall is often variable in magnitude for a particular level of rainfall. To determine further the causes of such variation, it may be necessary to investigate: (i) potential sources of contamination which may in themselves be episodic (e.g. manure and slurry spreading, livestock access to watercourses, intermittent discharges from combined sewage overflows etc.); and (ii) differential attenuation of faecal indicators and pathogens in soil and hill slope through-flow systems Well grounded empirical data on these processes is sparse and emerging research agendas suggest a need for such information by water quality and environmental regulators in the USA and the EU (Kay *et al.* 2006*b*, *c*, *d*). It is also notable that positive samples, particularly for *Cryptosporidium* and *Giardia*, were observed at some sites only during phase 2 of the study (i.e. the autumn). This may be due to the seasonal patterns of livestock management. Thus, additional sources of faecal material directly voided from livestock

Table 7 | Probabilities (p) associated with Spearman Rank Correlation analysis of the relationship between rainfall and bacteriological indicators or protozoan parasites

Site	Phase	Coliforms	*E. coli*	Enterococci	Clostridia	*Cryptosporidium*	*Giardia*
2	1	0.003	0.003	<0.001	0.027	n.s.	n.s.
	2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	all data	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
3	2	–	–	–	–	n.s.	n.s.
4	2	–	–	n.s.	n.s.	n.s.	n.s.
5	1	<0.001	<0.001	0.002	n.s.	0.022	n.s.
	2	<0.001	<0.001	<0.001	0.002	n.s.	n.s.
	all data	<0.001	<0.001	<0.001	<0.001	0.003	0.005
6	2	0.001	<0.001	0.001	0.012	n.s.	0.001
7	1	<0.001	<0.001	0.001	0.002	n.s.	n.s.
	2	0.001	<0.001	0.036	<0.001	0.001	<0.001

n.s. not significant (i.e. $p > 0.05$).

during the spring-summer-autumn period when livestock are grazing may be implicated in the greater number of positive results observed during phase 2. Higher geometric mean faecal indicator organism concentrations in surface waters during the summer period, when the majority of livestock is out to pasture, compared to the winter periods when cattle are housed, have been observed elsewhere (Rodgers *et al.* 2003; Dickson *et al.* 2005; Kay *et al.* 2006*a*).

CONCLUSIONS

The common assumption that treated commercial private water supplies are less likely to be polluted than the more numerous small supplies serving domestic properties may be unfounded (Fewtrell & Kay 1996; Shepherd & Wyn-Jones 1997; Fewtrell *et al.* 1998; Shepherd 2000; Said *et al.* 2003).

Indeed, the microbial concentrations of the supplies examined responded to environmental drivers, such as rainfall, in a very similar manner to other 'environmental' waters such as streams and field drains. This strongly suggests that the supplies were closely connected to their hydrological catchments where episodic but normal events such as rainstorms will dominate microbial movement and raw water concentrations. Thus, an understanding of the receiving catchment is vital in siting and exploitation of a new private water supply. It is also vital that any treatment installed has the capacity to treat the water during the episodes of poor water quality which will occur as part of the normal catchment microbial dynamics. This highlights the need for sampling raw water quality during events to inform the design of an appropriate treatment capacity at each supply.

These empirical data add weight to the comments of the UK HPA researchers quoted above which note the potential for health risks to transient populations supplied by such systems (Said *et al.* 2003).

Although some statistical relationship was found between faecal indicator organisms and the presence of other pathogens, the use of FIOs in assessments of regulatory compliance do not appear to provide a robust measure of public health risk, i.e. indicator absence does not preclude pathogen presence.

Table 8 | Probabilities associated with Spearman Rank Correlation analysis of the relationship between bacteriological indicators and protozoan parasites

Site	Phase	Coliforms	*E. coli*	Enterococci	Clostridia
Cryptosporidium					
2	All	<0.001	<0.001	0.002	<0.001
4	2	–	–	n.s.	n.s.
5	All	0.009	0.010	0.005	n.s.
6	2	0.024	0.042	0.004	0.014
7	1	n.s.	0.001	n.s.	n.s.
	2	<0.001	<0.001	0.029	<0.001
Giardia					
2	All	<0.001	<0.001	<0.001	<0.001
4	2	–	–	n.s.	0.001
5	All	0.046	n.s.	0.003	0.008
6	2	<0.001	<0.001	<0.001	<0.001
7	1	<0.001	<0.001	<0.001	0.008
	2	<0.001	<0.001	0.011	<0.001

n.s. not significant (i.e. $p > 0.05$).

Pathogen contamination of these supplies was highly episodic and the regulatory monitoring system employing relatively infrequent faecal indicator enumeration (e.g. class D Category 1 supplies (25 to 100 persons) sampled once per year) would be unlikely to characterise risks from this pattern of contamination. Thus, event-based monitoring of water at the consumer tap is essential to successfully characterise the potential risks associated with the contamination of private commercial supplies.

This adds weight to the call for a move to a 'risk assessment' paradigm in the regulation of private water supplies similar to that being proposed for regulated public supplies and recreational waters by WHO. This study provides the type of 'ground truth' microbiological data required for the design of such a regulatory risk assessment system and the acquisition of parallel data for a broader range of supplies should be considered to provide the 'evidence base' for the design of a risk assessment approach (Reid *et al.* 2004; WHO 1999, 2003, 2004).

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