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Cryptosporidium surveillance: investigations on the Loch Katrine water supply to Glasgow

S. J. Robertson, G. Bell, K. A. Punter and F. Reid

ABSTRACT

The upland water supply from Loch Katrine to Glasgow was unfiltered at the time of investigations reported here. Continuous monitoring of *Cryptosporidium* oocysts in water treatment works final water entering supply was a regulatory requirement from 2000, using a sampling and analysis protocol for low turbidity water that filtered 1,000 litre samples at site. *Cryptosporidium* surveillance was extended across the supply by developing a continuous monitoring programme for operational surveillance of raw waters, using the same sampling and analysis protocol. During 2001–2004 six final water samples exceeded 0.1 oocysts per 10 litres, but none exceeded 1 oocyst per 10 litres. *Cryptosporidium* 90th and 95th percentiles for source waters from three catchments were significantly lower for April–June than each of the other quarters ($p < 0.001$). Higher *Cryptosporidium* counts were generally observed in autumn and winter. The first two years following sheep removal from two catchment areas produced no clear impact on source water *Cryptosporidium* counts. High intensity rainfall in a 2002 storm led to marked increase in source water *Cryptosporidium* counts. A new water treatment works will provide a barrier to *Cryptosporidium* oocysts from late 2007.

Key words | *Cryptosporidium*, Loch Katrine, rainfall, sheep farming, water catchment, water supply

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INTRODUCTION

The nineteenth century introduction of a water supply from Loch Katrine to the city of Glasgow prevented further waterborne epidemics of cholera and typhoid. Those public health benefits were considerable, but scientific progress in the late twentieth century brought recognition that this unfiltered supply was exposed to potential contamination by protozoa of the genus *Cryptosporidium*, with species pathogenic to humans (Xiao *et al.* 2004) and other vertebrates including sheep and cattle.

The life cycle of *Cryptosporidium* (Smith *et al.* 2005) is complex but infection follows ingestion of oocysts, the transmissible and environmentally robust form of the parasite excreted in large numbers by infected hosts. Human infection can be acquired through several transmission routes, including contact with farm animals, person-to-person contact, food, recreational water, and the water supply (Meinhardt *et al.* 1996). The potential for water supplies to transmit *Cryptosporidium* oocysts rapidly to whole communities has attracted wide attention, although limited data on source water oocyst trends have been published.

Cryptosporidium oocysts are highly resistant to chlorine, at doses used for drinking water disinfection, and are normally removed by a physical barrier such as sand or membrane filtration. A new water treatment works for the Loch Katrine supply, programmed for completion in late 2007, is designed to remove colour and to remove *Cryptosporidium* oocysts.

In the area of the former Greater Glasgow Health Board, three quarters of the population were supplied with

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Loch Katrine water when a cryptosporidiosis outbreak in spring 2000 was attributed to that supply. From 90 confirmed cases *C. parvum* was identified in 57 of 60 faecal samples genotyped (Outbreak Control Team 2001). Human cryptosporidiosis cases have generally been associated with two species, *C. hominis* or *C. parvum* (Leoni *et al.* 2006), from the 16 species currently recognised (Sunnotel *et al.* 2006).

Reported here is a description of the Loch Katrine water supply and development of surveillance to assess *Cryptosporidium* prevalence across that supply. Results from 2001–2004 are summarised, source waters compared with the adjacent Loch Lomond supply, and seasonal variation investigated. The impact of exceptional rainfall on *Cryptosporidium* counts is reported through an associated water quality incident.

THE LOCH KATRINE WATER SUPPLY

During work reported here responsibility for this supply lay initially with West of Scotland Water (WoSW), one of three authorities formed in 1996 to take on water services from 12 former Regional and Island Councils. Further reorganisation in April 2002 led to the merger of North, East and West authorities to form Scottish Water, retaining water services under public ownership in Scotland.

Source waters

Loch Katrine lies some 50 km north of Glasgow (Figure 1) and is the principal source for the Glasgow water supply. Loch Arklet reservoir augments the supply by a gravity flow into Loch Katrine 2 km west of the intake for two aqueducts which convey water to Milngavie waterworks located 12 km north of Glasgow city centre. Glen Finglas reservoir further augments the supply by a gravity flow into Loch Katrine near its eastern extremity.

Table 1 shows features of the three source water catchments of the Loch Katrine supply, with features of the Loch Lomond catchment shown for comparison.

Water temperature (measured at Milngavie waterworks) typically ranges from 4°C in February to 17°C in August.

Catchment activity

Glasgow Corporation (a predecessor of Glasgow City Council) established and then operated the Loch Katrine supply from 1855 until 1968, purchasing the catchments of lochs Katrine and Arklet in 1920 to secure control over catchment activity. The status of direct water-authority control of catchment activity continued until 2005 when a land-management lease was let to Forestry Commission Scotland (Scottish Executive 2005).

About 150 cattle previously kept on the catchments of lochs Katrine and Arklet were all removed by 2000. Phasing out of sheep farming on these two catchments began in 2001; adult sheep had typically numbered 11,000 giving a stocking density of 1 per hectare ($100\,km^{-2}$), with about 6,000 lambs produced each year, delivered on the hillside. Each autumn most male lambs were sold, and ewe lambs were moved for wintering on to lowland pasture. In 2001, lamb production was reduced to 2,260, but a UK foot and mouth disease outbreak (Royal Society of Edinburgh 2002; Strachan *et al.* 2003) brought animal movement restrictions which delayed the return of ewes (wintered off these catchments) from April until August 2001. In 2002, 1,400 lambs were born but all sheep were removed from these two catchments between May and October of that year.

The Glen Finglas catchment had an estimated adult sheep stocking density of 0.6 per hectare ($60\,km^{-2}$), and about 90 head of cattle (Woodland Trust Scotland 2006). The considerably larger Loch Lomond catchment (Table 1) with multiple land ownership prevents accurate stock estimates, but sheep farming prevails on western catchment hills with cattle on lower lying lands to the south east.

Rainfall

Long-term (1971–2000) annual average rainfall at Stronachlachar, Loch Katrine, is 2,284 mm. Rainfall was above average in 2002 (2,613 mm) and 2004 (2,421 mm), and below average in 2001 (1,800 mm) and 2003 (1,817 mm). Seasonal rainfall patterns (Figure 2) show long-term monthly average rainfall was lower during April–August than winter months, but for 2001–2004 lower monthly average rainfall extended from March to September.

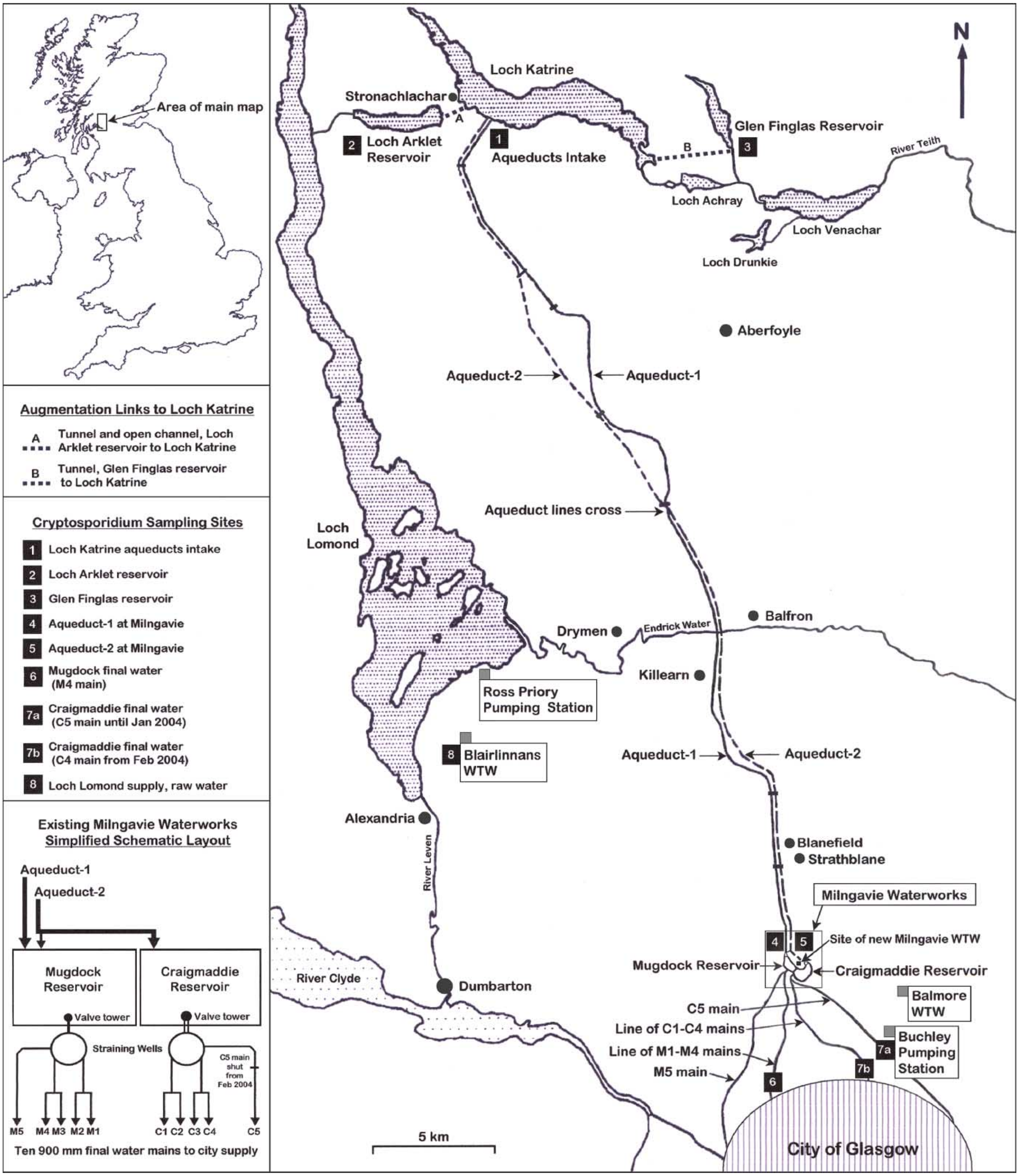

Figure 1 | General layout of the Loch Katrine water supply, also showing *Cryptosporidium* sampling sites and key installations on the Loch Lomond supply. Note that in February 2004 Buchley Pumping Station was transferred from the C5 main on the Loch Katrine supply to water supplied from Balmore WTW.

Table 1 | Catchment features of the Loch Lomond supply and features of the three catchments of the Loch Katrine supply

Parameter	Loch Lomond	Glen Finglas reservoir	Loch Arklet reservoir	Loch Katrine	Loch Katrine supply total
Catchment max. elevation (m AOD)	1,128	879	694	869	–
Catchment area (km^2)	769	39	18	94	–
Water area (km^2)	71.2	1.4	2.2	13.2	–
Top water level (m AOD)	7.9	157.0	145.2	115.3	–
Available storage (Ml)	87,100	19,100	12,300	64,600	96,000
Available yield (Ml d^{-1})	455*	82	60	343	485
Typical water turbidity (NTU)	0.7	1.0	0.8	0.4	–
Typical water colour (Hazen)	18	32	20	15	–

*Maximum permitted abstraction.
AOD = Above Ordnance Datum.
Hazen = True colour (0.45 μm filtered) measured on Platinum/Cobalt scale.

Aqueducts and Milngavie reservoirs

Water gravitates from Loch Katrine to Mugdock and Craigmaddie reservoirs at Milngavie via two aqueducts (Table 2). These aqueducts provide the sole supply to Mugdock and Craigmaddie reservoirs, which have capacities of 2,490 megalitres (Ml) and 3,190 Ml respectively.

Existing waterworks

Original treatment provided at Milngavie waterworks comprised straining wells, on the outlet from each of Mugdock and Craigmaddie reservoirs, housing fixed screens (aperture size 500 μm) to prevent fish and coarse debris entering supply.

The first chemical dosing system was chlorination, introduced in 1940. Lime-dosing was provided from 1978, supplemented from 1989 by dosing orthophosphoric acid (an orthophosphate source), to reduce plumbosolvency in lead service pipes used at many pre-1970 properties (Richards *et al.* 1980; Watt *et al.* 1996).

During 2001–2004 waterworks final water pH was normally controlled to pH 8.6; free chlorine was controlled to 1.8 mg l^{-1} in winter, reducing to 1.2 mg l^{-1} in summer when risk of imperfect disinfection performance was lower.

The existing waterworks has no treated water storage. Ten 36″ (900 mm) diameter trunk mains convey final water into the city, each of the Milngavie reservoirs normally supplying five trunk mains. The fifth Craigmaddie trunk main (C5) was designed for gravitational flow to Buchley pumping station (Figure 1) which pumps 50 Ml d^{-1} to seven

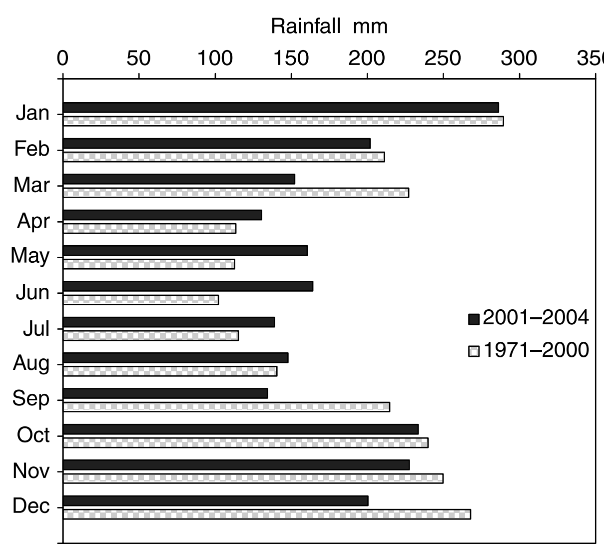

Figure 2 | 2001–2004 average monthly rainfall, and long-term (1971–2000) average monthly rainfall, at Stronachlachar, Loch Katrine.

Table 2 | Features of the two aqueducts that convey water from Loch Katrine to Milngavie waterworks

		Length of aqueduct sections by construction type, km				
	Max. Flow (Ml d^{-1})	Tunnels	Pipelines	Cut & Cover	Total	Average gradient (m km^{-1})
Aqueduct-1	190	21.0	6.0	14.5	41.5	0.158
Aqueduct-2	360	30.5	5.2	2.0	37.7	0.182

service reservoirs in higher districts of northern and eastern Glasgow.

In 2001, waterworks typical demand was 400 Ml d^{-1} from industry and a population of some 700,000 in the greater Glasgow area.

Waterworks redevelopment

The new water treatment works (WTW) at Milngavie will provide a direct filtration process (coagulation followed by rapid gravity filtration) and include treated water storage. The new WTW will thereby introduce a barrier to *Cryptosporidium* oocysts, reducing risk to public health from this parasite.

An initial phase of filtered water introduction into the Loch Katrine supply area began in February 2004, when treated water from Balmore WTW replaced the C5 supply to Buchley pumping station thereby reducing demand on Milngavie waterworks by 50 Ml d^{-1}.

Until February 2004 aqueduct-1 typically supplied 170 Ml d^{-1} into Mugdock reservoir; aqueduct-2 supplied 190 Ml d^{-1} into Craigmaddie reservoir and augmented flow into Mugdock reservoir by some 40 Ml d^{-1}. Following the 50 Ml d^{-1} reduction in demand in February 2004, operational changes were made whereby aqueduct-2 typically carried 350 Ml d^{-1} to supply both Mugdock and Craigmaddie reservoirs. Aqueduct-1 was then available to supply Loch Katrine water to Balmore WTW via a new gravity link at Strathblane, with a corresponding pumping reduction from Loch Lomond. This change provided removal of *Cryptosporidium* oocysts from aqueduct-1 water at Balmore WTW.

Loch Lomond water supply

Ross Priory pumping station pumps Loch Lomond water to Balmore WTW and Blairlinnans WTW (Figure 1), works with design capacities of 364 Ml d^{-1} and 91 Ml d^{-1} respectively. Redevelopment of both works was completed in 2000, with direct filtration replacing micro-strainers installed by the former Central Scotland Water Development Board in the 1960s.

SURVEILLANCE PROGRAMME DEVELOPMENT

Water quality regulation

The Water (Scotland) Act 1980 (HMSO 1980) is the primary legislation which requires water supplied for domestic purposes to be wholesome, now defined for public supplies through the *Water Supply (Water Quality) (Scotland) Regulations 2001* (Scottish Executive 2001), which implement European drinking water directive 98/83/EC (Council of European Communities 1998). Those Regulations incorporate the general requirement that water supplies contain no substance or microorganism that may be a danger to human health, but specific *Cryptosporidium* requirements are set out separately in Scotland through Directions.

The Cryptosporidium *Direction 2000* (Scottish Executive 2000*a*) (the 2000 Direction) incorporated into a regulatory framework measures relating to monitoring and control of *Cryptosporidium* in drinking water, including those recommended by Bouchier (1998). Water authorities were required to complete risk assessments of each supply, using a scoring system which estimated risk of *Cryptosporidium* being present in drinking water. Supplies categorised as high-risk required continuous monitoring of *Cryptosporidium* on water treatment works final water entering supply, from May 2000, using appropriate sampling equipment and methods of analysis described in *Guidance Notes* (Scottish Executive 2000*b*). Continuous monitoring required a stream of sample water at the sampling site to be passed through an appropriate collection device, which was a reusable Genera Technologies Limited Filta-Max housing (a filter-housing) containing a disposable Filta-Max compressed-foam filter module, at a minimum flow of 40 litres per hour, for each 24-hour sample.

The Cryptosporidium (*Scottish Water*) *Directions 2003* (Scottish Executive 2003) (the 2003 Directions) replaced the 2000 Direction. Prevailing sampling on the Loch Katrine supply was unchanged, but more flexibility on the type of regulatory sampling equipment was permitted.

The Water Services Unit of the Scottish Executive enforced drinking water quality regulations on behalf of Ministers until 2002, when the Drinking Water Quality Regulator (DWQR) was established to carry out this role independently of Ministers.

There is no regulatory maximum limit in Scotland for *Cryptosporidium* oocysts in drinking water, but the 2003 Directions require detection of oocysts in any sample of final water entering supply to be communicated to relevant Health Boards, local Councils, and the DWQR.

Regulatory sampling units

The 2000 Direction risk assessment scored the Loch Katrine supply as high-risk, and regulatory sampling units were installed on Mugdock and Craigmaddie final waters. Sampling units were supplied by Hydraulic Modelling Services Limited (HMSL) and comprised two cabinets. The sampling cabinet contained a diaphragm pump and associated equipment to measure and control flow (to 1 litre per minute) through the filter-housing as described elsewhere (Scottish Executive 2000*b*). The control cabinet housed a programmable logic controller, to control sampling and to activate alarms for events which included low flow across the filter-housing. Alarms were transmitted by telemetry (a 2000 Direction measure) to the Glasgow 24-hour control room.

Operational sampling units

Genera-based sampling and analysis offered the potential to monitor *Cryptosporidium* in Loch Katrine supply raw waters, if acceptable 24-hour sample volumes could be realised in practice.

Final water turbidity from Milngavie waterworks, typically 0.4 NTU, was higher than most filtered waters. However, it was anticipated that the tendency for proposed raw water sampling sites to show transient periods of increased turbidity might further diminish *Cryptosporidium* sample volumes. In practice, early sample volumes for Milngavie waterworks final waters were largely above 1,300 litres, encouraging development of a low-cost in-house *Cryptosporidium* sampling unit for operational (non-regulatory) raw water sampling on the Loch Katrine supply. This sampling unit, described in outline below, later became known as a Mullin Board unit, reflecting the name of the late craftsman who fabricated and installed early units and its compact (board-mounted) form.

The sample line was connected to the Mullin Board unit inlet, which was connected via 15 mm copper pipe to board-mounted equipment in the following sequence (a) strainer housing with replaceable 0.8 mm aperture strainer (b) 24-volt diaphragm pump (c) filter-housing connected via flexible hose and Fittings Specialisten (Denmark) stainless steel quick-connect couplings (d) rotameter ranged 0.2–1.8 litres per minute (e) water meter to BS 5728 class D (f) Maric valve to control flow to 1 litre per minute over a pressure range of 1–10 bar (100–1,000 kPa). Pressure gauges ranged 0–10 bar (0–1,000 kPa) were connected upstream and downstream of the filter-housing. The diaphragm pump was powered from an in-house designed and assembled transformer unit (230 volt to 24 volt), housed in a case protected to IP65 (Instrument Protection code 65 of the International Electrotechnical Commission standard 60529) and mounted on the Mullin Board.

Pathogens laboratory

The Glasgow pathogens laboratory of the predecessor Strathclyde Regional Council Water Department gained NAMAS (National Measurement Accreditation Service) accreditation for *Cryptosporidium* analysis in 1994. At that time about 1,200 *Cryptosporidium* samples were analysed annually, based on a method using Cuno cartridge filters (Standing Committee of Analysts 1989).

A sampling and analysis protocol for low turbidity water, with a notional detection threshold of 1 oocyst per 1,000 litre sample, was introduced in 1999 initially to monitor Loch Lomond final water at Blairlinnans WTW. This protocol subsequently became the regulatory protocol (Scottish Executive 2000*b*). In 2001 the pathogens laboratory was audited and formally approved by the Scottish Executive for regulatory analysis; in practice this was operating to the same standards (Drinking Water Inspectorate 1999) as in England & Wales and Northern Ireland.

After the Glasgow pathogens laboratory took on *Cryptosporidium* water sample analysis for Scotland as a whole, annual samples increased to 11,900 by 2003 (Scottish Water 2004). Subsequent closure of the Glasgow laboratories led to establishing *Cryptosporidium* analysis at Scottish Water's Edinburgh laboratories in 2004.

Sampling logsheet

Water meter readings for Genera-based samples were originally recorded on plastic evidence bags used to convey filter-housings from sampling site to laboratory. Due to anticipated challenges of achieving adequate raw water sample volumes, readings were also sought to identify changes in rotameter flow rate and head-loss (across filter-housings) over the sampling period. To record these readings, plus water meter readings for sample volume calculation, a logsheet was devised and printed on water-proof, tear-resistant, white paper, with yellow carbon copies for sampling site retention. Space was included to record filter-housing serial number, plus date and time received by pathogens laboratory analysts. A small writing surface was provided at all sampling sites to assist logsheet completion. Preceding yellow copies helped to investigate any low sample volumes.

The Glasgow in-house print-room made up logsheet pads with a unique customer reference number printed on each logsheet pair (white and yellow copies) to aid enquiries between colleagues. The logsheet was initially used for Loch Katrine supply operational samples, but extended to regulatory samples in 2001 after agreement with the Scottish Executive Water Services Unit.

METHODS

Regulatory sampling programme

Sampling from any one of the waterworks final water mains carrying Mugdock water (M1 to M5 mains) or Craigmaddie water (C1 to C5 mains) into supply was deemed to represent *Cryptosporidium* derived from the respective reservoir. Continuous monitoring progressed on the M4 and C5 mains from May 2000. When Balmore WTW replaced the C5 supply in 2004 the Craigmaddie final water sampling site was changed to the C4 main, using a Mullin Board unit rather than the HMSL unit due to space constraints at the new site.

Regulatory results are considered here for 2001–2004.

Operational sampling programme

The first Mullin Board unit was commissioned in August 2000 to sample water at Loch Katrine aqueducts intake; at this site the quality of water entering aqueduct-1 and aqueduct-2 was deemed the same. By May 2001 Mullin Board units were installed on both aqueducts to ensure that sampling continued if either aqueduct was out of service for maintenance; the two sampling units were not run concurrently.

All other operational sampling sites used Mullin Board units, where continuous monitoring began (a) in October 2000 on aqueduct-1 at Milngavie and aqueduct-2 at Milngavie (b) in March 2001 on Glen Finglas reservoir dam discharge (c) in April 2001 on Loch Arklet reservoir compensation-water discharge (d) in December 2000 on source water entering Blairlinnans WTW, on the separate Loch Lomond supply.

Results for these six operational sampling sites are considered here for 2001–2004.

Sampling at Glen Finglas reservoir was normally reduced to three 24-hour samples a week when water transfer to Loch Katrine was not operating. Loch Lomond source water sampling reduced to one 24-hour sample a week from March 2004.

Sampling procedure

Regulatory and operational sampling followed the same procedure. For each sample, the serial number of a clean filter-housing (a new filter module in a filter-housing) was recorded on the sampling logsheet, before connection to the sampling unit using quick-connect couplings. The water meter reading was recorded before starting flow through the sampling unit, when rotameter flow rate, and inlet and outlet pressures (or inlet pressure and headloss on HMSL units) were recorded. At the end of the 24-hour sampling period the same parameters were recorded again; the used filter-housing was removed from the sampling unit and taken to the laboratory in a sealed evidence bag along with the sampling logsheet.

Logsheet guidelines advised sample volume calculation before leaving site, to allow any obvious meter-reading anomaly to be checked. Initially, five filter-housings were dedicated to each sampling site.

Cryptosporidium sample analysis

Analysis procedures used to enumerate *Cryptosporidium* oocysts in operational or regulatory water samples are summarised below, and described in detail elsewhere (Scottish Executive 2000b).

Analysis methods

Analysis comprised elution and concentration of material captured on the filter module, followed by immunomagnetic separation (IMS) of oocysts from other debris captured. The inoculum from the IMS stage was stained (a) with anti-*Cryptosporidium* fluorescein-labelled monoclonal antibody, to stain the oocyst wall and (b) with 4′,6-diamidino-2-phenylindole (DAPI), to stain sporozoite nuclei within the oocyst.

Quantitative analysis was carried out by immunofluorescence (IFA) microscopy, to examine slides for objects which exhibited the size, shape and fluorescence characteristics of *Cryptosporidium* oocysts.

Objects were confirmed as oocysts by a combination of DAPI staining characteristics and differential interference contrast (DIC) microscopy.

Cryptosporidium counts were expressed as oocysts per 10 litres, and reported without adjustment for recovery, following prevailing UK conventions.

Analysis quality assurance

Internal quality assurance included the following controls:

1. Daily stain integrity and analyst counting controls: a suspension adjusted to give 80 to 120 oocysts per slide was used to inoculate control slides. Three control slides were stained and counted at the start of each day. With each batch of samples one control slide was stained as a positive control, and oocyst-free reverse osmosis (RO) water inoculated onto a slide was stained as a negative control.
2. Weekly IMS controls: a 100-oocyst flow cytometry-enumerated spike (±5%) was added to a blank IMS test and the sample processed to completion. Percentage recovery was plotted to show trends; typical IMS recovery was 75%, ranging from 60% to 90%.
3. Weekly whole method controls: a clean filter-housing was inoculated with a 100-oocyst flow cytometry-enumerated spike (±5%), by pumping the spike suspended in 10 litres of RO water through the filter. The filter was processed as a normal sample, and the count was recorded as a percentage recovery. Typical recovery was 40%, ranging from 30% to 50%.

External quality assurance was provided by participation in the UK-wide CRYPTS scheme (LGC 2002), administered by LGC Limited (the now independent Laboratory of the Government Chemist) in conjunction with the Scottish Parasite Diagnostic Laboratory (Stobhill Hospital, Glasgow). This scheme assessed laboratory performance once a month, covering counting controls, IMS controls and whole method controls. The pathogens laboratory also participated in the LEAP (Laboratory Environmental Analysis Proficiency) scheme.

Data handling and statistical methods

From eight sampling sites 10,377 *Cryptosporidium* results are considered here. During a 2002 water quality incident (below) consecutive 12-hour operational samples were taken from Loch Katrine aqueducts intake, aqueduct-1 at Milngavie, and aqueduct-2 at Milngavie. Those results have been transformed to 24-hour results by combining oocyst counts and sample volumes from consecutive pairs of 12-hour samples.

Charts were drawn using Microsoft Excel 2000 software. *Cryptosporidium* percentiles were compared between different sets of results, using Insightful S-Plus 7 software, by the Frisch Newton interior point method of quantile regression. Confidence intervals (95%) were produced for 90th and 95th percentiles, with p-values testing whether percentiles were significantly different between compared sets of results.

RESULTS AND DISCUSSION

Annual summaries

Table 3 presents annual summaries of *Cryptosporidium* results for each sampling site.

During 2001–2004 no sample of Mugdock or Craigmaddie final water exceeded 1 oocyst per 10 litres. No Craigmaddie final water sample exceeded 0.1 oocysts per 10 litres, but six Mugdock final water samples exceeded 0.1

Table 3 | Annual summaries of *Cryptosporidium* results, for source water on the Loch Lomond supply and seven sampling sites on the Loch Katrine supply

Sampling Site	Year	Number of samples	Median sample volume (litres)	Crypto-positive samples	Summary of high range values (oocysts per 10 litres)		
					90th percentile	95th percentile	Maximum
Loch Lomond*	2001	348	1,208	41%	0.036	0.049	0.213
	2002	354	938	55%	0.077	0.120	1.842
	2003	360	491	44%	0.071	0.093	0.381
	2004†	90	1,155	78%	0.063	0.080	0.206
Glen Finglas reservoir	2001	261	1,035	61%	0.123	0.230	2.178
	2002	317	947	50%	0.082	0.182	4.485
	2003	326	758	49%	0.100	0.184	0.980
	2004	248	877	74%	0.189	0.378	0.916
Loch Arklet reservoir	2001	237	767	39%	0.042	0.072	0.196
	2002	343	1,021	37%	0.030	0.053	0.255
	2003	356	917	23%	0.023	0.043	0.341
	2004	350	801	61%	0.055	0.076	0.643
Loch Katrine aqueducts intake	2001	332	1,324	36%	0.018	0.029	0.117
	2002	355	1,360	35%	0.020	0.026	0.677
	2003	346	1,364	23%	0.014	0.022	0.107
	2004	359	1,378	58%	0.024	0.035	0.137
Aqueduct-1 at Milngavie	2001	363	1,182	35%	0.024	0.036	0.174
	2002	336	1,143	48%	0.052	0.116	11.209
	2003	325	1,177	30%	0.021	0.037	0.214
	2004‡	31	–	–	–	–	–
Aqueduct-2 at Milngavie	2001	358	1,277	30%	0.017	0.023	0.253
	2002	361	1,033	41%	0.028	0.046	1.326
	2003	358	1,213	32%	0.018	0.033	0.120
	2004	360	1,272	50%	0.025	0.035	0.107
Mugdock final water	2001	365	1,282	27%	0.014	0.018	0.049
	2002	365	1,232	32%	0.013	0.021	0.353
	2003	365	1,142	19%	0.009	0.010	0.045
	2004	359	1,282	26%	0.010	0.016	0.032
Craigmaddie final water	2001	364	1,269	21%	0.008	0.015	0.040
	2002	365	1,179	28%	0.009	0.017	0.055
	2003	360	1,155	21%	0.009	0.010	0.038
	2004	360	1,177	28%	0.011	0.016	0.032

*Raw water entering Blairlinnans WTW.
†Sampling frequency reduced from seven to one 24-hour sample a week from March 2004.
‡Routine operation of aqueduct-1 to Milngavie discontinued from February 2004.

oocysts per 10 litres during a water quality incident (below). The highest final water annual 95th percentile was for Mugdock in 2002 (0.021 oocysts per 10 litres).

All raw water sampling sites showed their highest annual proportion of crypto-positive samples (samples with detected *Cryptosporidium* oocysts) in 2004.

Lower median sample volumes at Loch Arklet reservoir, Glen Finglas reservoir and Loch Lomond reflect a greater tendency for transient periods of raised turbidity (data not shown) than at Loch Katrine aqueducts intake, Mugdock final water or Craigmaddie final water. The low median sample volume for Loch Lomond in 2003 was largely attributable to diaphragm pump malfunction in July and August that year.

In 2004, observed 90th and 95th percentiles were higher than in the previous three years at Loch Katrine aqueducts intake, Loch Arklet reservoir, and Glen Finglas reservoir. The first two years following sheep removal (from Loch Katrine and Loch Arklet catchments in 2002) therefore showed no clear impact on *Cryptosporidium* prevalence, although longer-term trends will need careful review.

Overview of results

Figure 3 provides an overview of raised *Cryptosporidium* prevalence, based on 2001–2004 aggregated results for each sampling site.

Glen Finglas reservoir showed the greatest prevalence of raised *Cryptosporidium* results, with a 95th percentile (0.242 oocysts per 10 litres) almost an order of magnitude higher than the 95th percentile for Loch Katrine aqueducts intake (0.028 oocysts per 10 litres). Glen Finglas reservoir also has a greater tendency than the other sources for colour and turbidity to rise after heavy rain (data not shown).

Both 90th and 95th percentiles for aqueduct-1 at Milngavie were significantly higher ($p < 0.001$) than corresponding percentiles for Loch Katrine aqueducts intake, but neither 90th nor 95th percentiles for aqueduct-2 at Milngavie were significantly different than those for Loch Katrine aqueducts intake. This difference between the two aqueducts reflects a greater tendency for surface water ingress into aqueduct-1 along its route from Loch Katrine to

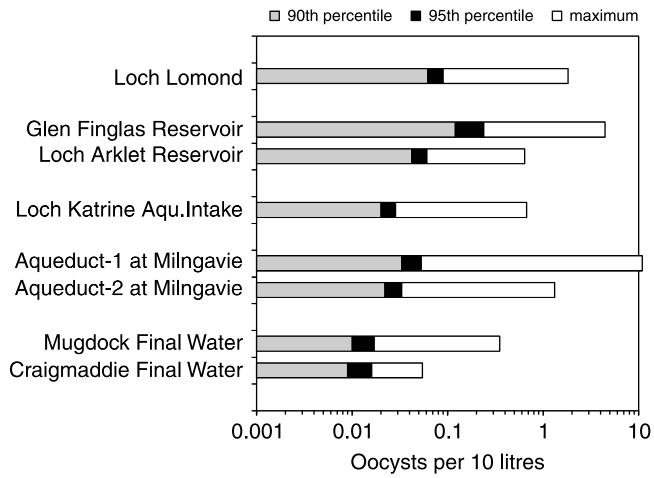

Figure 3 | An overview of raised *Cryptosporidium* prevalence, based on 2001–2004 results aggregated for each sampling site. Note that for Aqueduct-1 at Milngavie the maximum result was 11.2 oocysts per 10 litres, and routine operation of the same aqueduct to Milngavie was discontinued from February 2004.

Milngavie, due to the older age of aqueduct-1 and its greater proportion of cut-and-cover construction (Table 2). However, aqueduct-1 was not operated to Milngavie during most of 2004, preventing sampling, but 2001–2003 aqueduct results reflect findings from 2001–2004 except that the 95th percentile for aqueduct-2 at Milngavie in 2001–2003 was significantly higher than that for Loch Katrine aqueducts intake ($p < 0.05$), reflecting some surface water ingress.

Figure 3 also shows lower *Cryptosporidium* results in Mugdock and Craigmaddie final waters compared with aqueduct-1 and aqueduct-2 at Milngavie. For example, the 95th percentile for Craigmaddie final water (0.016 oocysts per 10 litres) was significantly lower ($p < 0.001$) than the 95th percentile for aqueduct-2 at Milngavie (0.033 oocysts per 10 litres) that supplied Craigmaddie reservoir (Figure 1).

Reduction in oocysts across the Milngavie reservoirs was probably due to dilution, but sedimentation needs consideration. Low sedimentation rates have been reported for oocysts unattached to other particles, 0.03 md^{-1} (Medema *et al.* 1998) and 0.023 md^{-1} (Dai & Boll 2006), although other suspended particles present are reported to increase oocyst sedimentation rate to that of the other particles through attachment (Searcy *et al.* 2005). Mugdock and Craigmaddie reservoirs have maximum depths of 15.3 m and 12.8 m and nominal residence times of 12 days and 16 days respectively. However, marked short-circuiting across these reservoirs was observed in earlier chemical tracer investigations, showing travel times of 14 hours and 20 hours for water to first reach Mugdock and Craigmaddie valve towers respectively after entering each reservoir (McAlpine & Robertson 1998). Opportunity for oocyst settlement in the Milngavie reservoirs therefore seems limited.

Seasonal variation

Figure 4 shows 60-day hind-cast moving-average rainfall, and 60-day hind-cast moving-proportion of crypto-positive results from the Loch Katrine aqueducts intake, for 2001–2004. Chart values for each date refer to the 60-day period up to and including that date; this chart therefore also utilises results from the last 59 days of 2000 to show 60-day trends from 1 January 2001.

The proportion of crypto-positive results tended to fall during spring and summer, and increase in the autumn to reach 60-day maxima in early winter. A partial exception to this is apparent for 2002, when there was no rise in crypto-positive results in autumn or winter; this followed a marked rise in *Cryptosporidium* results in summer 2002 after exceptional rainfall on 30 July which led to a water quality incident (below). Unusually large numbers of oocysts transported into Loch Katrine from that storm may have depleted the number of oocysts on the catchment in autumn that year.

Figure 5 summarises seasonal variation of raised *Cryptosporidium* prevalence, based on 2001–2004 results aggregated by quarter, for the three source waters of the Loch Katrine supply and for Loch Lomond source water. The highest 90th and 95th percentiles were from July–September, except for Loch Lomond where highest 90th and 95th percentiles were from January–March. Lowest 90th and 95th percentiles were from April–June for all four source waters.

Significance testing of this seasonality shows that 90th and 95th percentiles from July–September were both significantly higher ($p < 0.05$) than corresponding annual percentiles (percentiles based on results from all four quarters) for Loch Katrine aqueducts intake, Loch Arklet reservoir and Glen Finglas reservoir. For Loch Lomond the 90th percentile from January–March was significantly higher ($p < 0.05$) than the annual 90th percentile. For Loch Katrine aqueducts intake the 90th and 95th percentiles from July–September were both significantly higher than corresponding percentiles from each of the other quarters ($p < 0.05$), but not for the other three sources.

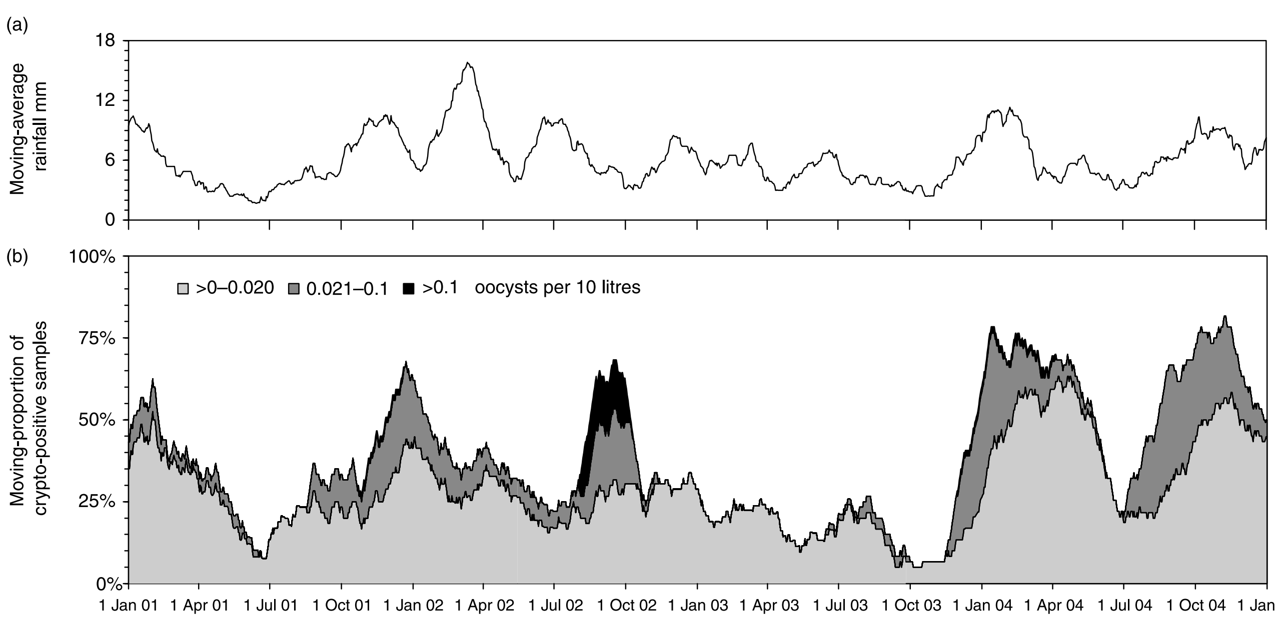

Figure 4 | Rainfall at Stronachlachar, Loch Katrine, and the prevalence of crypto-positive samples at Loch Katrine aqueducts intake (a) 60-day hind-cast moving-average of daily rainfall (b) 60-day hind-cast moving-proportion of crypto-positive samples, categorized in three ranges of oocyst concentration.

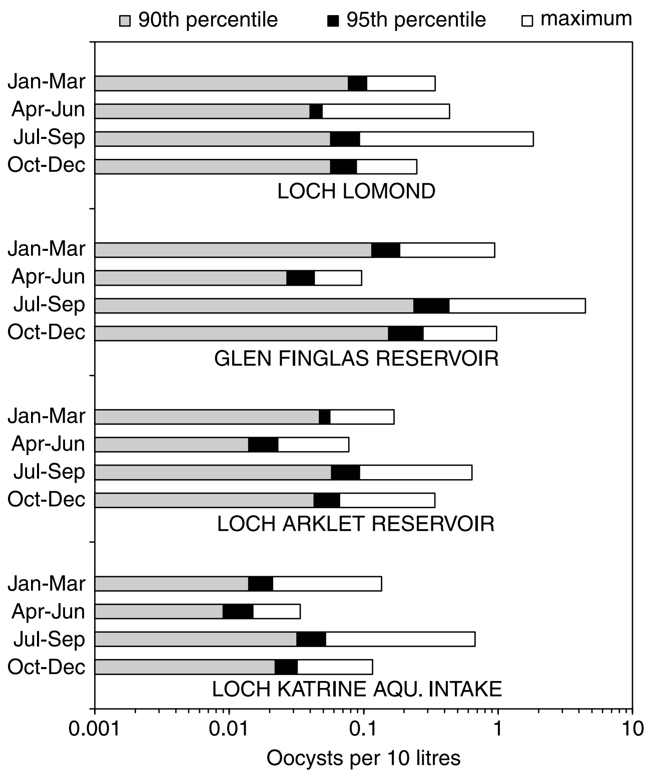

Figure 5 | Seasonal variation of raised *Cryptosporidium* prevalence, for source water of the Loch Lomond supply and the three source waters of the Loch Katrine supply. Based on 2001–2004 results aggregated by quarter.

The 90th and 95th percentiles from April–June were both significantly lower than corresponding annual percentiles for all four source waters ($p < 0.005$). The 90th and 95th percentiles from April–June were also both significantly lower than corresponding percentiles from each of the other quarters for Loch Katrine aqueducts intake, Loch Arklet reservoir and Glen Finglas reservoir ($p < 0.001$). For Loch Lomond, 90th and 95th percentiles from April–June were both significantly lower ($p < 0.005$) than corresponding percentiles from the January–March quarter only.

Lower 90th and 95th percentiles from April–June do not appear to be explained only by lower rainfall, because rainfall lower than winter months extended from March–September in 2001–2004 not just April–June (Figure 2). This needs further research, particularly if spring brings increased oocyst deposition onto catchment areas from newborn livestock. Programmes to speciate *Cryptosporidium* isolates from source waters would help to weigh the potential public health significance of seasonal variation in oocyst counts.

Water quality incident

Exceptional rainfall on 30 July 2002 led to raised turbidity in water entering Mugdock reservoir valve tower on 31 July. Precautionary operational changes were made immediately to supply three of the five Mugdock final water mains from Craigmaddie reservoir, reaching the normal hydraulic limit on the Craigmaddie supply. This reduced a 380,000 population supplied with Mugdock water to 140,000 supplied by M3 and M4 mains.

An Incident Management Team (IMT) was convened, as part of the Waterborne Hazard Incident Plan previously established by Scottish Water and stakeholders that included Health Boards and local Councils. *Cryptosporidium* results available to the IMT on 3 August included 11.2 oocysts per 10 litres in a 732 litre sample collected on 31 July from aqueduct-1 at Milngavie, with Mugdock final water entering supply reaching 0.353 oocysts per 10 litres on 2 August. The IMT issued a boil-water notice on 3 August, to customers supplied by M3 and M4 mains, which remained active until 7 August (IMT 2003). From 4 August Craigmaddie reservoir also supplied M3 and M4 mains, following extensive operational measures to then sustain unprecedented hydraulic demand on Craigmaddie reservoir. Mugdock reservoir was thereby withdrawn from service.

Figure 6 shows results for three sampling sites spanning the incident period. The continuous monitoring of raw water shows that the very high result from aqueduct-1 at Milngavie on 31 July did not originate from Loch Katrine aqueducts intake, suggesting surface water ingress had contaminated aqueduct-1 along its route between Loch Katrine and Milngavie.

Subsequently, results from Loch Katrine aqueducts intake increased to a maximum (0.677 oocysts per 10 litres) four days after the 30 July storm. This secondary source of raised *Cryptosporidium* delayed recovery of Mugdock reservoir (returned to service 21 August), although dilution across Craigmaddie reservoir constrained the maximum regulatory result for Craigmaddie final water to 0.05 oocysts per 10 litres.

Subsequent genotyping of *Cryptosporidium* isolates from the most contaminated water samples indicated that oocysts were mainly C. *andersoni*, a species considered non-pathogenic to humans; no clinical cases

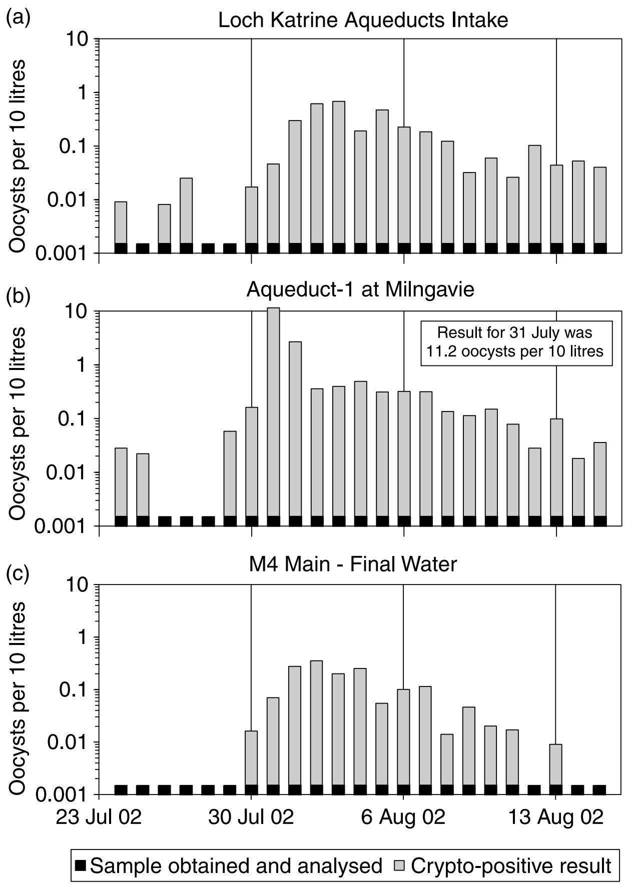

Figure 6 | *Cryptosporidium* results from three sampling sites, spanning the period of a water quality incident following high intensity rainfall on 30 July 2002. Note that chart dates refer to the end of each 24-hour sampling period, and that Craigmaddie reservoir supplied M4 main after 4 August.

of cryptosporidiosis were attributed to tap water during the incident (IMT 2003).

Analysis of the 30 July storm showed 16mm of rain fell in one ten minute period to the east of Glasgow; radar-estimated rainfall for Milngavie suggested 44.1 mm of that day's measured rainfall (52.4 mm) fell during 8 hours following midday, an event with an estimated 11-year return period (Meteorological Office 2002). Stronachlachar rainfall of 44.0 mm for 30 July was exceeded on 12 days during 2001–2004, but after none of those 12 days was there an increase in oocyst count at Loch Katrine aqueducts intake comparable to 2–5 August 2002. The impact of the 30 July storm therefore seems significant, and suggests that routine monitoring of rainfall intensity may help progress an understanding of oocyst mobilisation and transport into source waters.

Through this incident, raw water *Cryptosporidium* surveillance had indicated vulnerability of aqueduct-1 to oocyst contamination following intensive rainfall. Measures taken to reduce this risk to the supply included repairs to the fabric of aqueduct-1, and land drainage improvements adjacent to aqueduct sections identified by surveys to be vulnerable to surface water ingress. Operational contingency plans were also extended to include the option of emergency spilling of aqueduct-1 to watercourses if turbidity sharply increased.

CONCLUSIONS

Key findings from 2001–2004 investigations are summarised below:

- Raw water Genera-based sampling proved feasible on the generally low turbidity upland waters observed here, but transient raised turbidity had some adverse effect on sample volumes.
- The source with greatest prevalence of raised *Cryptosporidium* counts was Glen Finglas reservoir, with a 95th percentile of 0.242 oocysts per 10 litres.
- The prevalence of raised *Cryptosporidium* counts was lower in April–June than the rest of the year. Autumn and winter generally showed higher prevalence of raised *Cryptosporidium* counts.
- The first two years following sheep removal from Loch Katrine and Loch Arklet catchments showed no clear impact on source water *Cryptosporidium* counts.
- Aqueduct-1 at Milngavie showed greater prevalence of raised *Cryptosporidium* counts than aqueduct-2, reflecting its older age and tendency for surface water ingress along its route from Loch Katrine.
- *Cryptosporidium* counts reduced during flow across the Milngavie reservoirs.
- Six samples of final water entering the Glasgow supply exceeded 0.1 oocysts per 10 litres, but no final water sample exceeded 1 oocyst per 10 litres.
- Monitoring of rainfall intensity may help progress an understanding of mobilisation and transport of *Cryptosporidium* oocysts into source waters.

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