A time series study of drug sales and turbidity of tap water in Le Havre, France

Pascal Beaudeau, Alain Le Tertre, Abdelkrim Zeghnoun, Antonella Zanobetti and Joel Schwartz

ABSTRACT

The 80,000 inhabitants of the lower part of Le Havre obtain their water supply from two karstic springs, Radicatel and Saint-Laurent. Until 2000, the Radicatel water was settled when turbidity exceeded 3 NTU, then filtered and chlorinated, whereas the Saint-Laurent water was simply chlorinated. Our study aimed to characterize the link between water turbidity and the incidence of acute gastroenteritis (AGE). Records on drug sales used for the treatment of AGE were collected from January 1994 to June 1996 (period 1) and from March 1997 to July 2000 (period 2). Daily counts of drug sales were modeled using a Poisson Regression. We used data set 2 as a discovery set, identifying relevant (i.e. both significant and plausible) exposure covariates and lags. We then tested this model on period 1 as a replication dataset. In period 2, the daily drug sales correlated with finished water turbidity at both resources. Settling substantially modified the risk related to turbidity of both raw and finished waters at Radicatel. Correlations were reproducible in period 1 for water from the Radicatel spring. Timeliness of treatment adaptation to turbidity conditions appears to be crucial for reducing the infectious risk due to karstic waters.

Key words | France, gastroenteritis, medication sales, tap water, time series study, turbidity

Pascal Beaudeau (corresponding author)
Alain Le Tertre
Abdelkrim Zeghnoun
Institut de Veille Sanitaire,
14 rue du Val-d'Osne,
94415 Saint-Maurice,
France
E-mail: *p.beaudeau@invs.sante.fr*

Pascal Beaudeau
Antonella Zanobetti
Joel Schwartz
Harvard School of Public Health,
02215, Boston, MA,
USA

ACRONYMS AND ABBREVIATIONS

AGE	Acute gastroenteritis
ARMA	Autoregressive moving average
ATC	Anatomo therapeutic chemical
GAM	Generalized additive model
GP	General practitioner
IC95	95% Interval of confidence
NA	Not available (missing data)
NTU	Nephelometric turbidity unit
P50	Percentile 50 or median
P98	Percentile 98
RA	Attributable risk
RR	Relative risk
TSS	Time series study
WHO	World Health Organization

INTRODUCTION

Waterborne infection outbreaks have been recognized as a public health concern for almost two centuries and the methods for detection, investigation, surveillance and prevention are now well established. The role of sub-standard tap waters in the background incidence of infections of fecal origin has also been described (Zmirou *et al.* 1987). The case of tap water meeting quality standards in developed countries remains less clear for numerous reasons: (i) Resources and treatment process are decentralized, resulting in a large range of microbial quality of distributed waters, and consequently in a differing attributable disease burdens. Furthermore, risk may vary over time according to the episodic circulation of the numerous types of pathogens involved. The reproducibility of risk estimates is not

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straightforward from one site to another or even from one period of time to another for a given site. (ii) As the expected risk levels are rather low, powerful data sets must be collected to have hope of detecting any associations. (iii) The exposure to pathogens must be assessed through more sophisticated microbial indicators than conventional bacterial indicators (e.g. *Escherichia coli*), since they are completely inactivated by chlorination. (iv) In the cohort studies (Payment *et al.* 1991; Calderon & Craun 2006; Zmirou-Navier *et al.* 2006), the choice of the microbial approach to exposure requires multi-annual duration and high costs due to both the number of pathogens possibly involved and the variability of water contamination over time. A recent review stated that no attributable risk could be drawn from such studies, whatever the design (Craun & Calderon 2006).

Time series studies (TSS) represent an interesting alternative. A database set up for health care management can provide adequate indices for the incidence of acute gastroenteritis (AGE): for example, drug sales (Beaudeau *et al.* 1999), physician visits (Aramini *et al.* 2000), calls for medical advice (Gilbert *et al.* 2006) or visits to hospitals (Schwartz *et al.* 2000; Tinker *et al.* 2008) have already been used to form AGE case count indices. In the 1980s, water operators developed a water operation strategy based on the online measurement of chlorine concentration or turbidity as surrogates of exposure, in order to adapt treatment to variations of water quality in a timely way. To date, turbidity has been the more commonly used proxy for exposure to pathogens in TSS. Statistical methods developed in the field of air pollution epidemiology have been applied to track the endemic risk due to tap water. The benefit of this design is firstly the lower cost, as no specific data collection such as microbial analysis is needed. The study period may be extended at no additional cost. In addition, the population serves as its own control, as is made clear by the correspondence of the method to case-crossover analysis (Lu & Zeger 2007). Biases related to interviews are absent. Some limitations are also clear, such as the need for large populations to be included in the study, ranging from tens of thousands to hundreds of thousands of people, depending on the morbidity index sensitivity. Good traceability of water is also required to avoid misclassification on exposure, and the use of a surrogate such as turbidity inevitably introduces exposure error. The availability and quality of water data remain the practical challenge. A review of TSS concluded positively on the relevance of the approach and on the existence of a risk due to tap waters conforming to water quality standards (Mann *et al.* 2007).

The studies published to date have described water systems that are fed by surface water and simply chlorinated (Aramini *et al.* 2000), or previously clarified using different techniques: filtration (Tinker *et al.* 2008), coagulation-filtration (Egorov *et al.* 2003) or coagulation-flocculation-settling-filtration (Schwartz *et al.* 1997, 2000).

In common with other designs for analyzing waterborne illness, and in contrast to air pollution, the replication of TSS in other locations is problematic due to differences across locations in water sources and their quality, treatment methods, and variations in the degree to which turbidity can serve as a surrogate for pathogens. However, the opportunity to obtain long time series enables the use of other time periods in the same location for a replication sample, an approach which has hitherto been lacking.

The present study focused on a water system fed by karstic springs. Such groundwater aquifers are vulnerable to contaminated runoffs and subject to sharp and poorly foreseeable changes in the spring water quality. They are characterized by spikes of turbidity and fecal contamination. This dynamic challenges the operators who have to adapt the treatment process to muddy water occurrence very quickly (i.e. within a few hours).

The study periods ran from January 1994 to June 1996 (data set 1) and from March 1997 to July 2000 (data set 2). A first study based on data set 1 was published in 1999 (Beaudeau *et al.* 1999). Data were reanalyzed using new methods. A full report on the analysis of data set 2 is available (Beaudeau *et al.* 2010).

The objective of the study described here was to use the approach of discovery in one data set, and replication of the discovered model in a replication data set, to provide a stronger test of the causality of the identified association. Specifically, we model the risk associated with turbidity, taking into account the effect of adaptive treatment. The risk model obtained on data set 2 was tested on data set 1.

MATERIALS AND METHODS

The Le Havre water system and related data

Le Havre is a city of 200,000 people in Normandy, France. The climate in Le Havre is temperate, with regular precipitation throughout the year (600 mm per year and up to 1,000 mm on the plateau) and shows moderate seasonal variation in temperature.

Like most other water supply companies in this region, the city of Le Havre obtains its drinking water from the Eastern Normandy chalk aquifer. Radicatel and Saint-Laurent are the two springs that provide water to the 80,000 inhabitants of the lower city. Both are located in rural areas, where most of the population lives in villages of 500–1,500 inhabitants equipped with waste-water treatment plants, and the other inhabitants live in farms scattered throughout the countryside and use domestic septic tank systems with leaching fields. Population density in the watersheds is around 100 people per km^2 (Institut National de la Statistique et des Etudes Economiques 2004). Land use is shared between intensive agriculture and cattle farming with about 100 heads of cattle per km^2 (Institut National de la Statistique et des Etudes Economiques 2011). Rivers are scarce in the catchment area, as a karstic underground network drains water from the chalk aquifer. The density of sinkholes is about one per km^2 (Chemin *et al.* 1992). These sinkholes may be used to discharge treated waste waters. During heavy rain episodes, they may also swallow up polluted water from surface runoffs, as well as bypassed wastewater from sewage systems. As a result, a background fecal contamination is ever present in the spring waters and the concentration soars in heavy rain conditions: fecal coliforms averaged 78 CFU/100 mL (standard error = 226, $N = 1{,}064$) at Radicatel and 8 CFU/100 mL (standard error = 13, $N = 1{,}229$) at Saint-Laurent (Regulatory monitoring of the Health Ministry 1979–1998, N=2,500, unpublished data). Fecal contamination is strongly associated with turbidity spikes at Radicatel springs. Turbidity at Saint-Laurent springs is much lower than at Radicatel. The water temperature at the springs remains stable throughout the year (11–12 °C). The pH remains constant at around 7.0 during treatment, storage and transport.

At the time of the study, the Saint-Laurent plant was equipped with only chlorination facilities and its production capacity ranges from 15,000 to 35,000 m^3 per day. Two different feeders (phi900 and phi500) draining two different spring sets bear the water to the urban distribution system. The final chlorination targeted a 0.2–0.3 mg/L free chlorine residual. Water from phi500 was distributed without any storage, resulting in a chlorine contact time of 6 hours, whereas water from phi900 flowed through a storage tank that achieved over 12 hours of contact time before distribution.

The Radicatel water treatment plant was a conventional plant built in 1968 with facilities for coagulation-floculation-settling, rapid sand filtration and chlorination, and a capacity of 63,000 m^3 per day. Treated water from Radicatel flowed down a 30 km pipe and passed through two reservoirs, providing a residence time ranging from 1 to 2 days between the plant outlet and the first consumer's tap. Water was re-chlorinated at the second reservoir, ensuring a 0.2–0.3 mg/L free chlorine residual at the urban network inlet.

Both plants operated under low turbidity conditions and the Radicatel plant was used in a direct filtration mode (i.e. no coagulation-floculation-settling). When raw water turbidity exceeded 3 NTU at Radicatel, the plant was operated in full treatment mode. When raw water turbidity exceeded 1.5 NTU at Saint-Laurent, water was discharged to the river and the Radicatel plant took over the production of drinking water for the lower city.

The distribution network providing the lower city with drinking water was fully connected and fed roughly 50/50 by the two springs. However, the proportions varied substantially over time. As a consequence, the origin of water running at a given tap could vary from day to day.

In order to rapidly adapt treatment procedures to water quality turbidity changes, the plants were monitored continuously by an automated monitoring system that included effluent and influent (Radicatel) turbidity and free chlorine measurements. In period 1, measurements were missing for phi500. Turbidimeters (HACH®) were used, which provided a resolution of less than 0.01 NTU for distributed water and 0.1 NTU for raw water from Radicatel. Turbidimeters were cleaned and calibrated every month. Each significant hourly change in distributed water turbidity (>0.20 NTU) was verified by technical staff. Measurements were recorded every 15 minutes. Turbidimeters for finished waters were out of commission 8% (period 1) and 24% (period 2) of the time.

We checked the 15 minute turbidity measurements for spurious data with the help of the water operator. False spikes due to handling were removed and the drift in raw water turbidity caused by mud deposit over optics was corrected following a method detailed in Beaudeau *et al.* (2010). Daily means were then calculated or considered missing if more than 25% of the 15 minute measurements were missing or disabled.

The Saint-Laurent water discharge dummy covariate was drawn from the daily count of water volume produced (data not shown). When production data were not available (i.e. January–September 1998), days with instant turbidity over 1 NTU were assumed to have been discharged. As the operation staff kept no specific record, the Radicatel settling dummy covariate was estimated from the raw and treated turbidity record (Figure 1). MétéoFrance provided temperature data.

Drug sales data

The area served by the water treatment plants was covered by a surveillance network of pharmacists. This network provided a useful tool for surveillance through their sales records of medications.

The pharmacy included in the earlier study (Beaudeau *et al.* 1999) was used again for period 1. This pharmacy left the pharmacists' network before period 2. Five other pharmacies were selected for the second study, based on their location, the quality of the data and the profile of the customers. All recruited pharmacies were frequented by consumers from the neighborhood (2,000 customers per pharmacy in average), which limited the misclassification rate for exposure due to living outside the lower city to a rate below 10%, according to the pharmacists.

Medications were selected on the basis of both official directions for use and real field practice with the help of some local general practitioners (GP) and pharmacists. The list consisted of anti-emetics (classes A04A and A03F of the Anatomical Therapeutic Chemical (ATC) classification (WHO Collaborating Centre for Drug Statistics Methodology 2011)), pro-biotic anti-diarrhoeals (A07F), intestinal anti-propulsive (A07D, A07X), intestinal absorbents (A07B, A02X), intestinal anti-infectious agents (A07A E) and oral re-hydration salts.

The consistency of data slightly differed between data sets 1 and 2. In the 1994–1996 study, the list was not intended to be exhaustive, but it provided data for two-thirds

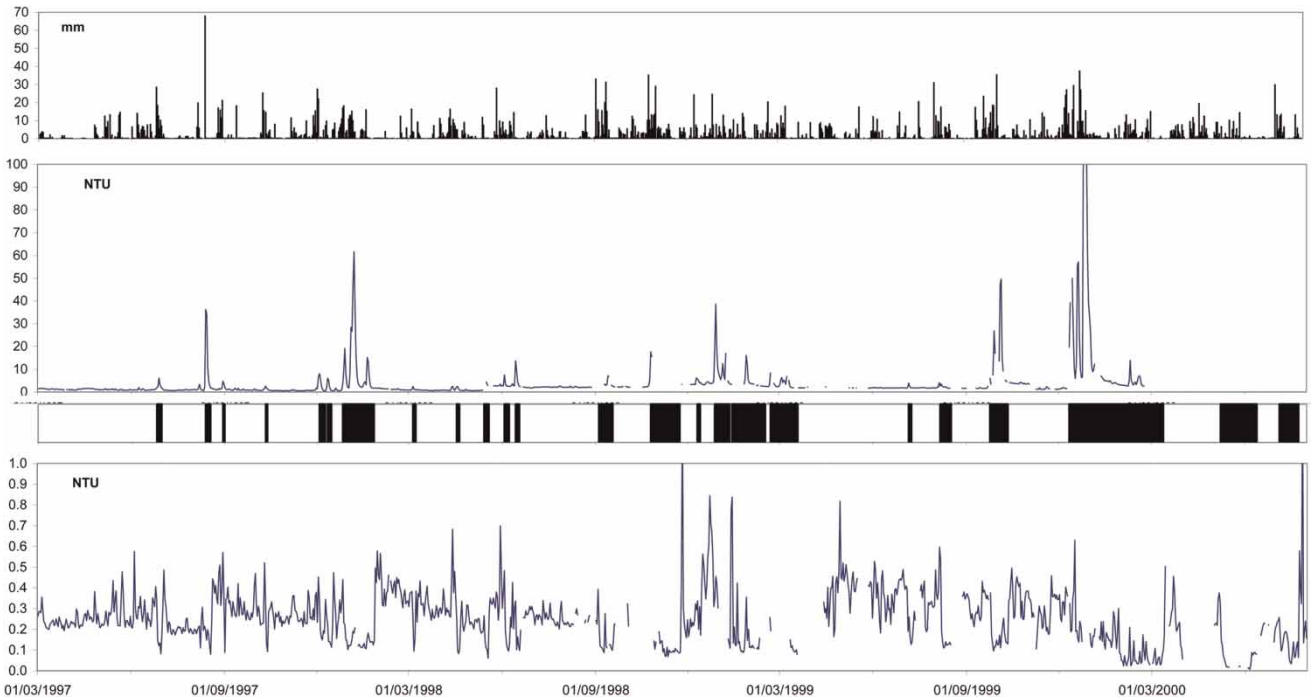

Figure 1 | Daily precipitation, turbidity of raw water, settling periods and turbidity of finished water at Radicatel (top down).

of the best selling products. These corresponded to about 90% of the total sales, expressed in number of boxes. The list was updated between the two study periods to cover all available products.

The more critical changes between the two studies concerned the data management by the pharmacists. In France, 99% of the resident population benefits from social funding of their health expenditure. Remote transmission of prescribed drug sales data to the 'Assurance Maladie' (health branch of the French social insurance system) for partial reimbursement became daily in period 2 instead of weekly in period 1. This evolution guaranteed true dating of sales figures in the second period. On the other hand, the use of just-in-time stock control, which determined the availability of over-the-counter sale data (about 10% of the boxes sold for AGE), spread significantly between the two study periods. The pharmacy included for period 1 was the only network pharmacy which ran its stock online at these times, whereas three of the five pharmacies recruited for the second period did so.

One month of data was missing for the 30-month first period. Forty-four monthly files out of a total of 205 were missing for the 41-month second period, either because of closures due to annual holidays (one third of the missing files) or failures in recording processing. As at least one pharmacy delivered valuable data for any given month of the period, we were able to infer counts according to the sales figures of the pharmacies, whether present or absent.

Statistical modeling

Generalized additive model

Generalized additive models (GAM) (Hastie & Tibshirani 1990) were built using the functions of the 'mgcv' package from the R program. Since the daily count of the boxes sold follows an overdispersed Poisson process, it was modeled using the quasi-Poisson distribution family and a log link function.

To test the association between drug sales and the covariates for non-linearity, we used penalized cubic splines (Eilers & Marx 1996). A penalized spline fits a nonlinear curve using separate cubic polynomials to model the association in different ranges of covariate value, e.g. four intervals for temperature and turbidity. A penalty term is then added to the log-likelihood that constrains the amount of nonlinearity. The optimal amount of constraint, and hence the optimal amount of nonlinearity (if any) may be determined by using generalized cross-validation, which approximates the model fit in replication data set.

Time-varying confounders, which stemmed from demographics, commercial and epidemiological features, were controlled through available covariates (Table 1): time trends and seasonality were modeled by using a penalized spline of the time index (day of study) (with five degrees of freedom per year of study), within month time pattern by using a cyclic penalized spline of the day of month, day of the week by dummy covariates and temperature, again using penalized splines. Auto regressive terms were added if necessary.

We then focused on the risk due to turbidity and investigated whether the use of enhanced treatment at Radicatel (including coagulation-flocculation-settling afterwards referred to as 'settling') changed the association between water turbidity and AGE.

Modeling strategy

The basic modeling strategy was to fit a model on data set 2 (the training set), which was the larger data set, and then examine how well that model predicted AGE in data set 1 (the replication data set). Because different pathogens have different latencies, and the type of pathogen typical in the water is not known, we had to explore multiple plausible lags between turbidity and drug sale, as well as potential nonlinearities. These were chosen based on goodness of fit and biological plausibility in the discovery dataset, and the chosen model was confirmed in the replication set. From the training step, we chose the relevant turbidity measurements and associated lags on both statistical and plausibility considerations. The criteria for eligibility were the following:

- significance of the coefficients or splines;
- consistency of the response over several consecutive lags;
- robustness of the response to the exclusion of extreme values, e.g. removal of the turbidity measurement over the percentile 98% (P98). When outliers caused spurious

Table 1 | Possible time confounders of the relationship between turbidity and the AGE drug daily sales

Time confounders	Source	Detail	Control
Trend	Commercial	Change in the population included	Penalized cubic spline of time (initialized on the base of 5 degrees of freedom per year)
Seasonal variations	Epidemiology	Winter viral outbreaks	
School vacations	Demographics	Change in the population included	Dummy covariates
Within month variations	Commercial	Cash availability of patients	Penalized cubic spline of day of month (initialized on the base of 3 degrees of freedom)
Days of the week	Commercial	Store closure	Sunday sales treated as missing; factor covariates for the other days
Holidays	Commercial	Shop closure	Treated as missing
Temperature	Epidemiology	Acting on both the exposure and the sensitivity to develop symptoms	Penalized cubic spline of daily mean air temperature (initialized on the base of 3 degrees of freedom)
Residual auto-correlation	Epidemiology	Contagion, secondary AGE cases	Auto regressive terms

unstable associations, we used restricted data sets (i.e. values <P98) for the ultimate expression of the risk;

- agreement with an expected adverse effect of turbidity in finished water. An increasing response function was required to be considered true. Also, linear response functions were chosen when non-linear spline functions did not bring substantial improvement to the model;
- likelihood of the lags used for the different turbidity covariates according to incubation delay for AGE.

The latency of the response of drug sales to turbidity covered: (i) the mean residence time of the water from the turbidity measurement point to the taps; (ii) the incubation period from exposure to the onset of symptoms; and (iii) the delay between the onset of symptoms and the purchase of drugs. The water residence time from the place of measurement to consumers' taps was 2–3 days for raw water turbidity at Radicatel and 1–2 days for other turbidity measurements. We considered a 1–10 day range for incubation duration, since it includes most of the incubation time distributions of the known pathogens causing AGE (Chin 2000). A specific field survey showed that the consultation with a GP and the purchase of the drugs required 1–2 days from the onset of symptoms (Bounoure *et al.* 2010). Hence the likely total delay for an effect of turbidity on drug sales ranged between 3 and 14 days (4–15 for raw water turbidity at Radicatel).

The models fit to data set 2 were then tested on the data set 1, keeping the same lags and time spans for exposure assessment.

RESULTS

Descriptive analysis

The average daily sales of the selected drugs were 8.1 boxes in data set 1 (one pharmacy) and 27.5 in data set 2 (five pharmacies pooled). Marked but irregular seasonal variations were observed, generally peaking in January and reaching twice the summer level in most years (1994, 1995, 1999 and 2000). Peaks corresponded to common viral winter outbreaks which are seen throughout Europe. No other outbreak patterns were observed. The distribution of daily sales (Table 2) shows a strong over-dispersion compared to a random Poisson process, with a variance reaching three times the mean.

A strong and irregular seasonal pattern was also apparent in the raw water turbidity (Figure 1 and Table 2). The time pattern for raw water turbidity consists of sharp spikes set on a fairly steady background level, typical of karstic springs (Beaudeau *et al.* 2001). Despite a similar shape, levels of both background and spikes are very different at Saint-Laurent (P50 = 0.1 NTU and P98 = 0.3–0.5 NTU) and Radicatel

Table 2 | Distribution of the variables. Saint-Laurent turbidity data corresponding to discharge are included

	NA%	Mean	Min	P2	P10	P25	P50	P75	P90	P98	Max
1994–1996 ($N = 911$)											
Drug sales (1 pharmacy)	16	8.1	0	0	2	4	7	11	16	20	32
Temperature (°C)	0	11.0	–2.1	–0.2	4.1	6.8	10.5	15.0	18.3	22.9	26.5
Turbidity Saint-Laurent phi900 (NTU)	14	0.13	0.05	0.07	0.08	0.09	0.1	0.14	0.18	0.35	1.22
Turbidity Saint-Laurent phi500 (NTU)	100	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Turbidity Raw Water Radicatel (NTU)	6	4.0	0.9	1.3	1.5	1.9	2.4	3.2	6.6	23.3	70.2
Turbidity Raw Water Radicatel – no settling (NTU)	6	2.3	0.9	1.3	1.5	1.7	2.1	2.6	3.0	4.7	18.0
Turbidity Raw Water Radicatel – settling (NTU)	4	8.6	1.1	1.8	2.6	3.1	5.3	8.1	19.2	43.2	70.2
Turbidity Finished Water Radicatel (NTU)	3	0.34	0.03	0.05	0.07	0.23	0.34	0.45	0.58	0.79	1.46
Turbidity Finished Water Radicatel – no settling (NTU)	3	0.42	0.12	0.21	0.28	0.32	0.38	0.49	0.60	0.80	1.46
Turbidity Finished Water Radicatel – settling (NTU)	5	0.12	0.03	0.04	0.05	0.06	0.08	0.12	0.24	0.50	1.10
1997–2001 ($N = 1{,}250$)											
Drug sales (5 pharmacies)	0	27.5	0	0	0	15	27	38	51	70	124
Temperature (°C)	0	11.8	–1.6	1.5	5.8	8.1	11.6	15.8	17.9	21	25.6
Turbidity Saint-Laurent phi900 (NTU)	27	0.11	0.05	0.06	0.07	0.08	0.09	0.11	0.16	0.33	1.36
Turbidity Saint-Laurent phi500 (NTU)	29	0.10	0.01	0.02	0.04	0.05	0.07	0.10	0.16	0.46	0.90
Turbidity Raw Water Radicatel (NTU)	26	4.0	0.5	0.6	0.7	1.0	1.8	2.9	5.4	31.5	229.0
Turbidity Raw Water Radicatel – no settling (NTU)	22	1.6	0.5	0.6	0.7	0.9	1.4	2.0	2.7	4.1	7.0
Turbidity Raw Water Radicatel – settling (NTU)	36	10.8	0.5	1.3	2.1	2.7	4.1	7.5	24.8	61.7	229.0
Turbidity Finished Water Radicatel (NTU)	16	0.25	0.01	0.04	0.10	0.17	0.24	0.33	0.41	0.53	1.37
Turbidity Finished Water Radicatel – no settling (NTU)	16	0.30	0.05	0.13	0.20	0.23	0.28	0.36	0.43	0.56	1.37
Turbidity Finished Water Radicatel – settling (NTU)	16	0.14	0.01	0.02	0.04	0.09	0.13	0.17	0.26	0.44	0.84

(P50 = 2–3 NTU and P98 = 23–32 NTU). The winter turbidity episodes were very strong during some winters (e.g. 1998–99, maximum turbidity at Radicatel >200 NTU) or almost absent (e.g. 1995–96, 1999–2000, maximum turbidity at Radicatel <10 NTU) according to the weather conditions.

The marked rise in turbid spike frequency from period 1 to period 2 (e.g. P98 = 23 versus 32 NTU at Radicatel) may have been a result of the combined effect of rainier winters during period 2 and an adverse land use evolution due to increasing urbanization (Saint-Laurent catchment area) and the plowing of former meadows. The background turbidity of the spring waters improved from period 1 to period 2 at Radicatel (medians: 2.4 versus 1.8, respectively), but slightly deteriorated at Saint-Laurent (phi900: 0.10 versus 0.11). The reasons for this development remain unclear.

Due to treatment fluctuation, the turbidity of Radicatel finished water experienced more day to day variations than raw water. Treated water turbidity at Radicatel was paradoxically much lower when the raw water turbidity was higher, because the full treatment was then in operation (21% of the time in period 1 and 30% in period 2). For instance, in data set 2, the medians were 0.23 and 0.09 NTU, respectively. Splitting the turbidity data according to settling conditions also showed that the operation management improved between the two study periods. More turbid spike starts were missed in the first period (maximum daily raw water turbidity off-settling: 18 NTU in period 1 versus 7 in period 2), and the increasing attention paid to treatment adaptation impacted all Radicatel turbidity statistics (Table 2). Saint-Laurent water discharge into the river was rare in the first period (<2% of the time with a maximum turbidity of 1.5 NTU), but increased to 7% of the time in the second period, even though the revised limit of 1 NTU was never reached.

Turbidity effect on AGE

We first fit the model for the covariates listed above, and confirmed the plausibility of the results. Drug sales decreased by 1.6% (IC95 = [1.3%; 1.9%]) per additional °C of 24 hour mean air temperature, and increased in the winter as expected.

We then examined cubic splines of finished water turbidity, for the lags 3–15 and two data set options (all turbidity data included versus data below quantile 98). The turbidity covariates were introduced separately. These results (Table 3) indicated that:

- the effect of the turbidity at Saint-Laurent was very significant for both phi500 and phi900. When significant, the shapes of the spline functions were monotonically increasing, except for phi500 for which the shape was slightly decreasing up to 0.04 NTU;
- the Radicatel raw water turbidity was associated with a strong, monotonic increase in AGE with exposure;
- the Radicatel finished water turbidity showed a similar pattern but with only poor significance;
- the most significant results and steepest dose response curves were observed between lag 6 and 11, but earlier response and, more frequently, later responses occurred. For instance, we did not observe weakening in the Radicatel raw water turbidity response up to the 15th lag.

On this basis, we decided to focus on further steps on lags 6–8 which represented the optimal tradeoff between significance and epidemiological plausibility, and led to consistent outcomes over the different sources; we also expected that the extension of the span to 3 days would provide more stable and accurate risk values.

The introduction of non-linearity in the response of drug sales to turbidity did not bring clear improvement. In the training step, the improvement was significant on Saint-Laurent's phi900 (p <0.01) but only marginal for phi500 (p <0.1), and the shape of the function was not far from a straight line (Figures 2 and 3). However, the improvement in fit from a nonlinear fit was not reproducible on data set 1 (replication step). Furthermore, the few microbiological studies performed on karstic systems did not suggest a

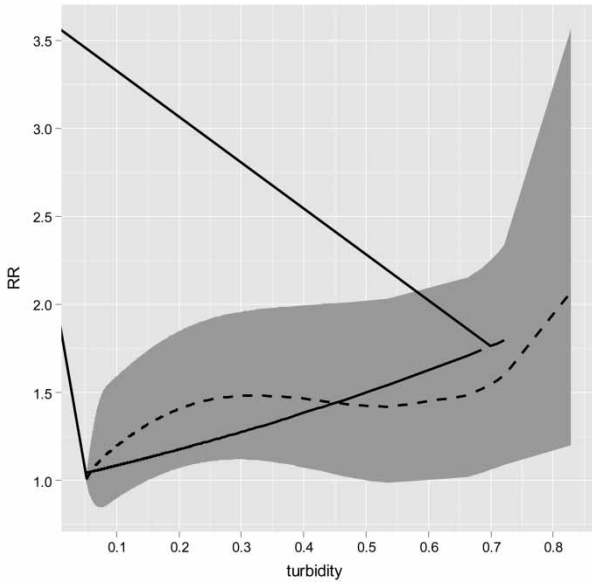

Figure 2 | Relative risk function for the turbidity at Saint-Laurent phi900. B-spline using 4 degrees of freedom (dotted line and its IC95) versus linear function (solid line) adjusted for the 6–8 lag mean turbidity.

Table 3 | Test of turbidity covariates expressed as spline functions in the GAM, according to different lags and two data set options (all data included versus data below quantile 98).

Variable		Lag (days) 3	4	5	6	7	8	9	10	11	12	13	14	15
Turbidity Saint-Laurent phi900 (NTU)	All data	***	***	**	***	***	***	***	***	***	***	***	***	**
	< Quantile 98	***	***		***	***	***	***	***	**	**	*	***	*
Turbidity Saint-Laurent phi500 (NTU)	All data	***	***	***	***	***	***	***	***	***	***	***	***	***
	< Quantile 98	***	***	***	***	***	***	***	***	***	***	***	***	***
Turbidity Raw Water Radicatel (NTU)	All data	*	*	***	***	***	***	***	***	***	***	***	***	***
	< Quantile 98	***	***	***	***	***	***	***	***	***	***	***	***	***
Turbidity Finished Water Radicatel (NTU)	All data					*		*	*			***	***	*
	< Quantile 98							*	**				*	

*p < 0.1; **p < 0.01; ***p < 0.001.
Light gray: spline function increasing in trend; dark gray: spline function strictly increasing.

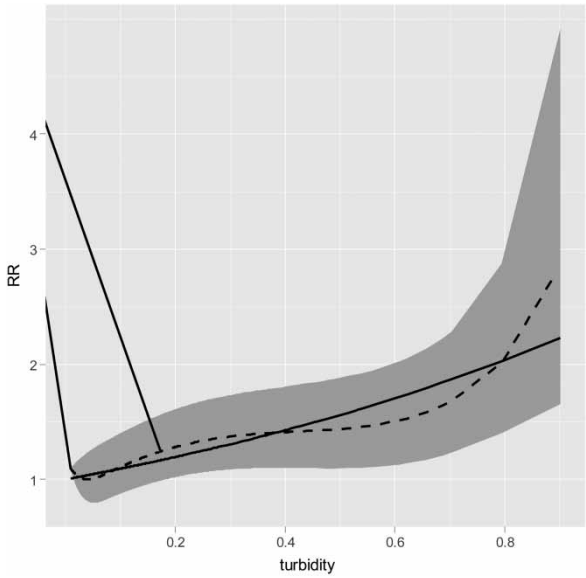

Figure 3 | Relative risk function for the turbidity at Saint-Laurent phi500. B-spline using 4 degrees of freedom (dotted line and its IC95) versus linear function (solid line) adjusted for the 6–8 lag mean turbidity.

nonlinear association (Dussart-Baptista 2003; Stadler *et al.* 2009). On the contrary, they showed that the fecal bacteria load remained roughly proportional to turbidity level throughout most turbidity episodes. This was demonstrated particularly well on the Radicatel's springs (Ville du Havre, unpublished results). We therefore used linear fits for turbidity in subsequent analyses.

Next, we introduced a dummy covariate for settling as a potential modifier of the effect of Radicatel turbidity on AGE risk. Consideration of this interaction dramatically improved the association of finished water turbidity with drug sales (p <0.0001). On days when extra treatment was not operating, there was a positive and significant (p <0.01) association with finished water turbidity. The interaction with raw water turbidity was also significant (p <0.0001). We did not express the Radicatel water turbidity as mean over 3 days, since it did not make sense given these operational issues. Indeed the triggering of settlement took only a 6-hour delay to achieve steady operation conditions and stops happened instantly. Therefore, the days including settling start up or switch off were classified ambiguously according to the settling dummy variable. By widening the span, the ambiguity would spread over three more lags: 25 and 24 settling operation periods in the study 1 and 2 would make questionable classification for 150 and 146 observations, respectively. Risk estimates are given for an inter-quartile variation of turbidity such as per NTU (Table 4).

The inter-quartile RR associated with the phi500 water turbidity was higher than others (1.13 versus 1.10 or less), in spite of a lower turbidity. Hence the excess risk (per 0.1 NTU) was very heterogeneous by source and the treatment: +27% for phi500, +23% for phi900, +5% for Radicatel filtered water and +12% for Radicatel settled water. The switch to settling was also associated with a step in the risk of +11% (p <0.1).

The evolution of linear estimates of risk according to the lag is given in Figures 3–5. Whatever the source, the risk spread over a large 3–15 day span of time showing a plateau, the location and width of which may varied somewhat by source: lag 11–15 for phi500 (Figure 4), 7–10 for phi900 (Figure 5), 13–15 for Radicatel filtered water (Figure 6) and 5–15 for Radicatel settled-filtered water (Figure 7). The inter-quartile risk associated with raw water turbidity at Radicatel was qualitatively consistent with the finished water-related risk, i.e. a significant positive effect modified by settling. The maximal risk was located on lag 9 in both operation conditions.

The above associations were partly reproducible on set 1 (Table 4). However, the association with the turbidity at Saint-Laurent phi900 became insignificant. Radicatel raw water turbidity correlated with drug sales (RR = 1.02; $p < 0.05$) if data were restricted to under P98 turbidity values and if no interaction with settling was entered. On the contrary, we found a correlation for treated water turbidity only when an interaction with settling was introduced (p <0.1). This correlation remained after restriction of the data set to non-extreme values of turbidity, i.e. below P99 and over P01 for both turbidities with and without settling. However, the slopes of the linear response functions were different between the two periods, as was the constant associated with the settling dummy covariate (Table 4).

DISCUSSION

Reliability of the turbidity related risk

The study aimed at highlighting and, if possible quantifying, the short-term effect of turbidity on drug sales fluctuations.

Table 4 | Relative risks of AGE drug sales related to turbidity. The reference time is the prescription day (lag=0)

Covariate	Lag (days)	1994–96 Interquartile range of turbidity (NTU)	Interquartile RR	Relative Risk[a]	1997–2001 Interquartile range of turbidity (NTU)	Interquartile RR	Relative risk[a]
Turbidity, Saint-Laurent, phi500	6–8	NA	NA	NA	0.05–0.10	1.134 [1.089; 1.181]	1.268 [1.178; 1.362]
Turbidity, Saint-Laurent, phi900	6–8	0.09–0.14	1.031 [0.960;1.107]	1.066 [0.918;1.238]	0.08–0.11	1.068 [1.048; 1.089]	1.233 [1.167; 1.300]
Turbidity, Raw Water, Radicatel	7–9	1.9–3.1	1.003 [0.993;1.013]	1.002 [0.994;1.013]	0.87–2.38	1.011 [1.007; 1.016]	1.007 [1.004; 1.010]
Turbidity, Raw Water, Radicatel no settling	8	1.7–2.6	1.022 [0.954;1.093]	1.027 [0.944;1.116]	0.9–2.0	1.022 [1.005; 1.039]	1.019 [1.004; 1.034]
Turbidity, Raw Water, Radicatel – settling	8	3.1–8.1	1.070 [0.871;1.316]	1.014 [0.972;1.056]	2.7–7.5	1.019 [1.012; 1.027]	1.004 [1.002; 1.006]
Settling	8			1.144 [0.954;1.371]			1.240 [1.122 ; 1.386]
Turbidity, Finished Water, Radicatel	6–8	0.24–0.44	1.001 [0.943;1.062]	1.000 [0.971;1.031]	0.13–0.27	1.028 [0.984; 1.072]	1.020 [0.989; 1.051]
Turbidity, Finished Water, Radicatel no settling	7	0.32–0.49	1.179 [1.142; 1.218]	1.103 [1.033; 1.177]	0.23–0.36	1.069 [1.030; 1.108]	1.054 [1.024; 1.085]
Turbidity, Finished Water, Radicatel – settling	7	0.09–0.17	1.025 [0.983; 1.069]	1.040 [0.974; 1.110]	0.09–0.17	1.094 [1.052; 1.137]	1.119 [1.066; 1.173]
Settling	8			1.354 [1.068; 1.717]			1.103 [0.960; 1.268]

[a]Units: Turbidity-related risks expressed per 0.1 NTU, except for Radicatel raw water turbidity (per NTU); Settling-related risks are unit less.

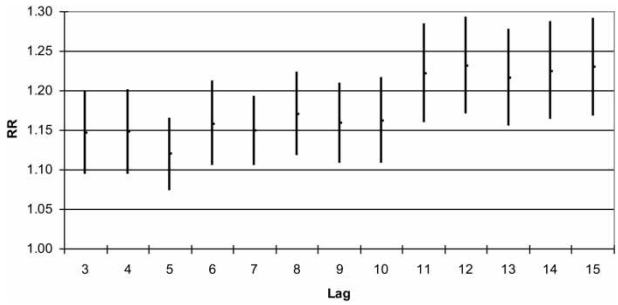

Figure 4 | Interquartile relative risk according to the lag (days), Saint-Laurent, phi500, data set 2.

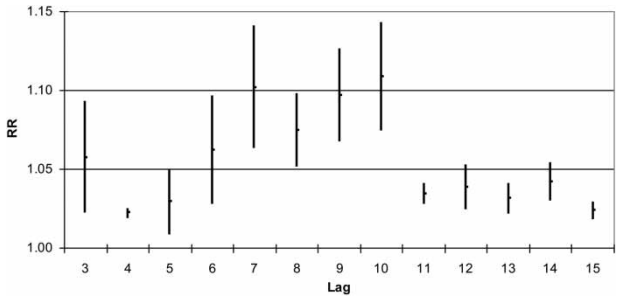

Figure 5 | Interquartile relative risk according to the lag (days), Saint-Laurent, phi900, data set 2.

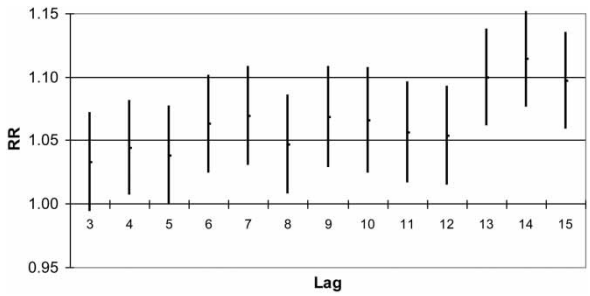

Figure 6 | Interquartile relative risk according to the lag (days), Radicatel without settling, finished water, data set 2.

The results obtained on data set 2 met both the predefined statistical criteria (i.e. significance, robustness to data and lag variations) and plausibility (i.e. consistency with expected adverse effect and incubation delay). Moreover, the modifying effect of the settling operation on the link between finished water turbidity and drug sales also argued for causality. Indeed, coagulation-flocculation-settling and direct sand filtration involve different physical processes which may lead to a lesser pathogen load for a similar load in raw water and for a similar turbidity level in finished water: 'Without proper chemical pretreatment [which was the case at Radicatel], rapid rate filtration works as a simple strainer and is not an effective barrier for microbial pathogens' (LeChevallier & Au 2004). Thanks to the use of GAMs instead of ARMA models (Beaudeau *et al.* 1999), this risk pattern was qualitatively reproducible on data set 1, which also provides a strong argument for causality.

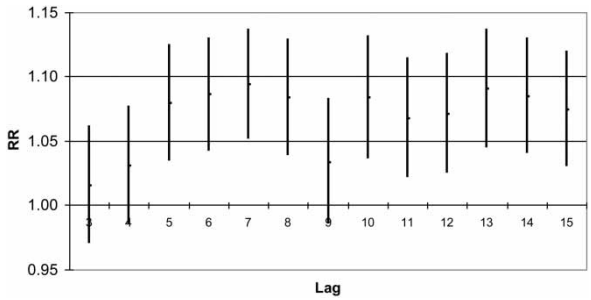

Figure 7 | Interquartile relative risk according to the lag (days), Radicatel with settling, finished water, data set 2.

The significance of risk estimates addresses random variations of the sample but does not prevent the possibility of bias and confounding effects detailed previously (Beaudeau *et al.* 2010). Here, we examine three main sources of bias, i.e. the restriction of the time window used for exposure assessment, the degree of control for trend and the seasonal variations and the multi-source supply of the lower city.

The response of AGE to environmental stimuli spread over a fairly long time span (e.g. 1 month), according to incubation delay distribution of the numerous pathogens involved in AGE. This required special attention to short-term confounder control, such as the weekly and monthly variations of drug sales, which may have created fake late positive lags. On the other hand, real late response could exist due to parasites, especially *Giardia* sp. We therefore focused on a mean incubation delay of 5 days, which we considered an acceptable tradeoff for all AGE pathogen incubation times. This restriction induced an underestimation of the associated risk.

Marked time patterns were present in both the target variable (AGE drug sales) and the proxy for exposure (water turbidity). The inclusion of a spline function of time achieved the control for trend and season variations of drug sales, especially the regular winter viral outbreak, which spread

primarily by direct contact and could confuse the AGE-turbidity relation through co-seasonality, in addition to the control for day of the month effects. On the other hand, the control had to remain smooth enough not to erase the secondary peaks which could denote potentially waterborne AGE clusters. We thus chose a more intensive smoothing of the spline of time than the optimal shape according to the generalized cross validation score. The definition of a method which would guide the control for trend in the field of waterborne AGE TSS would help stabilize risk estimates since these estimates are sensitive (in both directions) to the amount of smoothing put in the spline of time.

The water supply of the lower city from multiple resources brought potential misclassification on exposure and hence possible confusion and bias among the different risk estimates.

Missing data prevented us from explicitly addressing the multiple resource issue by entering several turbidity covariates in a 'multi-exposure' model. Indeed, by accumulating missing periods of all exposure covariates, 56% of the observations would be missing for fitting a multi-exposure model from data set 2. Furthermore, daily flows of distributed water in the study area data were missing, which prevented us from weighing turbidity covariates by the corresponding flows.

The turbidity levels at the different resources were not independent because the watersheds are close to each other and hence concurrently showered by the same rainfalls. Both settling at Radicatel and discharge at Saint-Laurent operated at high turbidity levels and sometimes overlapped. Whereas daily measurement of raw water turbidities correlated quite strongly between Radicatel and Saint-Laurent ($r = 0.76$–0.45), turbidities in finished waters were slightly and negatively correlated (max $r = -0.19$), due to the episodic switches of treatment at Radicatel. The mean turbidity level at Saint-Laurent was significantly higher when settling operated at Radicatel (0.17 versus 0.11 NTU, data set 1).

The direction and the strength of the bias on one given resource turbidity related risk resulting from the absence of control of other resources could be qualitatively characterized. Since risk functions were assumed to be linear, they can be defined by the slope coefficients, and a constant coefficient for settling. The bias onto slope coefficients of the finished water turbidity related-risks should be limited because these covariates correlated poorly (non-differential bias). On the contrary, the interpretation of the constant associated with the settling dummy covariate remained ambiguous. To our minds, the apparently adverse effect of settling was mainly attributable to the concurrent, and not controlled, degradation of the Saint-Laurent water quality.

Lastly, the bias was by far higher for period 1 than for period 2. Day-to-day fluctuations in the shares of distribution network supplied by the Saint-Laurent and Radicatel feeders may also prevent evidencing a true relationship between phi900 Saint-Laurent's water turbidity and AGE drug sales during period 1. In period 2, data were provided by 5 pharmacies located throughout the distribution zone. They covered about 20% of the people living in the studied area and likely represented sales to persons supplied by all the sources. In contrast, in period 1, only one pharmacy met the requirements for enrolment in the study. Consequently, the daily rate of misclassification on exposure ranged dramatically larger and reached 100% for the days when only one resource fed the pharmacy area.

The bigger misclassification rate in period 1 compared with period 2 is likely to have contributed to the poor reproducibility of the risk for Radicatel raw water and Saint-Laurent water turbidity on period 1.

Public health issues

At the local level, this study gave the water operation staff of Le Havre an insight into health risk attributable to the municipal water system, even if multi-resource feeding could have confused the interpretation of the risks. The regulatory water microbiological monitoring was unable to provide such information because of the lack of sensitivity of microbial indicators in finished chlorinated water. Furthermore, the expression of risk as a function of everyday operating conditions (turbidity, settling) helped support operation improvement. In the case of Le Havre, the high rate of missing data combined with multi-resource feeding hampered quantitative risk assessment. Thus, reducing missing data is a priority for the use of turbidity in epidemiological TSSs.

The risk values obtained for period 2 were substantially more reliable than for period 1 and could be converted into

attributable risks (AR = RR/(1–RR)), since there were major grounds in favor of causality and no consistent arguments against. The AR related to the finished waters of each resource were, however, not additive because they were drawn from regression models including only one exposure covariate, but each could be considered an underestimate of the overall risk.

We used these attributable risks to examine the potential to reduce the population disease burden using simulations comparing what actually happened to scenarios where finished water turbidity kept pre-defined limits. These simulations showed that simply filtered water from Radicatel caused the main share of avoidable risk. For example, if operators could hold a limit of 0.05 NTU in Radicatel produced water, it would result in a 15% (IC95=[4; 26]) reduction in risk of illness. Because of a possible bias due to the concurrent degradation of the Saint-Laurent water quality, this scenario should have overestimated the settling dummy covariate coefficient and consequently the disease burden attributable to Radicatel resource. Nevertheless, 15% can be considered a conservative global estimation of avoidable risk for both resources.

Practically, the settling could not be operated continuously at Radicatel, since particle retention by sludge bed clarifier did not perform properly when the raw water turbidity was below 3 NTU. In 2004, the water operator actually opted to change for lamella sludge bed clarifier which enabled settling to be continuously carried out. In 2007, filtration facilities were implemented at Saint-Laurent, with coagulation on filter when raw water turbidity exceeded 3 NTU. These improvements met the operational conclusions of this study and were probably able to achieve a substantial cut in the waterborne risk.

To date, published TSS have concerned water systems fed by surface water (Schwartz *et al.* 1997, 2000; Aramini *et al.* 2000; Egorov *et al.* 2003; Gilbert *et al.* 2006; Tinker *et al.* 2008). This study suggests that drinking water produced from karstic resources and meeting quality regulation requirements may also result in a significant disease burden. Because of the sudden variations in the particle load of the raw water which challenges treatment adaptation, karstic waters could be more at risk than water systems fed by large rivers or reservoirs, for which variations in particle load are smoother even though the mean load is higher.

Would the consumers judge acceptable a risk of about 15% of AGE attributable to tap water in developed countries? WHO guidelines (World Health Organization 1996) stated: '… for a pathogen causing watery diarrhea with a low case–fatality rate (e.g. 1 in 100,000), this reference level of risk [i.e. 10^{-6} disability-adjusted life-years (DALYs) per person per year] would be equivalent to 1/1,000 annual risk of disease to an individual'. This criterion, derived from a Quantitative Microbial Risk Assessment approach, was shaped to help assess new non-vital activities, e.g. the opening of a new bathing area. However, it does not help answer the question of the acceptable waterborne share of the global burden due to enteric pathogens. This requires the need for health targets adapted to an epidemiological syndromic approach to risk. Assuming the relevance of AGE as an index for fecal pathogen-related diseases, the index could be locally adapted to derive appropriate operational parameters to minimize the share of AGE attributable to tap water, or set an acceptable level of risk.

CONCLUSION

This study differs on two points from other published TSS focusing on the role of drinking water turbidity onto the background AGE risk.

The first is the cross testing of the model on a data set which was not used for fitting. Even though the weakness of these data limited the cross validation to qualitative appraisal, the testing validated the make-up of the turbidity-related risk at Radicatel, including the modifying effect of the settling.

The second original feature of the study is the karstic nature of the aquifer, whereas all other published studies focused on surface water. This study suggests that drinking waters produced from karstic waters could bear a specific risk. Indeed, even if the quality of raw water remains better than surface water for 90% of the time, risk concentrates on the periods of sharp changes in water quality, resulting in a challenging need for a treatment adaptation.

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