Evaluation and Development of *Cryptosporidium* Risk Assessment
Final Report
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Executive Summary

The Scottish Government require Scottish Water to monitor drinking water supplies at a frequency determined by a risk assessment score as detailed in the *Cryptosporidium* (Scottish Water) Directions 2003. The ultimate aim of the Directions is to reduce the risk of outbreaks of *Cryptosporidiosis* due to contamination of drinking water by targeting raw and final water monitoring effort to those sites that are considered to be highest risk. The Scottish Government commissioned ADAS and CREH to statistically validate and enhance the risk assessment approach by the identification and appropriate weighting of easily measured variables that maximise its predictive power.

Work Package 1 ensured that the project was set up effectively and that the programme of work met the requirements of the Scottish Government.

Work Package 2 – Data Collation and Validation

Cryptosporidium monitoring data

A total 24,205 water samples were collected by Scottish Water between January 2006 and October 2007, 3,776 (16%) of which were raw water samples and 20,249 (84%) final water samples. Of the raw water samples, 965 (26%) were positive for *Cryptosporidium*, with a maximum oocyst count of 0.73 L^{-1}. Of the final water samples, 1484 (7%) were positive, with a maximum oocyst count of 1.62 L^{-1}. A total of 304 final water supplies were monitored, 266 (87.5%) of which were sourced from surface water and 38 (12.5%) from groundwater, with 165 (54%) having at least one *Cryptosporidium* positive sample. Only 80 raw waters (surface waters) were monitored for *Cryptosporidium*, of which 65 (81%) had at least one positive sample.

Regional differences

There was a statistically significant difference in the proportion of sites that had positive final water between the four operational regions (Pearson's χ^2= 14.22, P=0.003), with the South East having the highest and the South West the lowest. The median final unweighted risk assessment score also varied significantly between regions (Kruskal-Wallis χ^2= 42.47, P<0.001), with the North East having the highest and the South West the lowest. The South West region did not have the lowest proportion of sites with positive raw water or lowest median catchment level risk assessment score, therefore the reductions in final risk assessment scores and positive samples in the South West may be due to improved water treatment.

Work Package 3 - Evaluation of the Cryptosporidium Risk Assessment Approach

Statistical power

Power calculations show that the sample size for final surface waters (n=266) will be sufficient to detect a 2-fold increase in risk of *Cryptosporidium* in the final water with 90% power, but the sample size for groundwaters (n=38) is insufficient assuming the same criteria. The sample size for raw surface waters (n=80) is sufficient to detect a 2.5-fold increase in risk of *Cryptosporidium* in the raw water with 80% power.

Performance of existing surface water risk assessment

Receiver operating characteristic (ROC) analysis shows that the surface water catchment risk assessment score is a poor predictor of *Cryptosporidium* occurrence in raw water. Using logistic regression, there was shown to be an increased odds of *Cryptosporidium*-positive raw water if the catchment was scored as medium risk (OR=2.33, 95% C.I. 0.34-15.95) or high risk (OR=5.56, 95% C.I. 0.65-47.84) compared to low risk, but these increased odds were not significant. Raw waters that were scored as medium risk had a significantly greater proportion of samples positive than low risk sites (b=2.16, z=4.20, P<0.001), as did those that were scored as high risk (b=1.95, z=3.80, P<0.001). These results suggest that using the proportion of samples positive may be a more accurate representation of risk than a binary outcome.

ROC analysis indicated that the overall unweighted risk assessment score is a fair predictor of *Cryptosporidium* occurrence in final water. Logistic regression demonstrated increased odds of final surface water being positive for medium risk sites (OR=5.21, 95% C.I. 2.85-9.54, P<0.001) and high risk sites (OR=75.85, 95% C.I. 10.04-572.77, $P<0.001$) compared to low risk sites. Final surface waters that were scored as medium risk had a significantly greater proportion of samples positive than low risk sites (b=2.11, z=17.36, $P<0.001$), as did those that were scored as high risk (b=2.52, z=21.55, $P<0.001$).

Limitations of the monitoring data

- A large proportion (73%) of WTWs do not have raw water monitoring data, which limits the validation and improvement of the catchment risk assessment score, however the sample size was deemed sufficient.
- To assess the performance of the treatment process it will only be valid to use data from sites that have positive raw water samples, since the effectiveness of the treatment can only be tested if challenged by oocysts in the raw water entering the treatment works. Alternatively, the improved catchment risk score could be used as a surrogate for the *Cryptosporidium* load in the raw water entering the WTW.
- The likelihood of detecting a contamination event will be dependent upon the frequency of sampling, both due to a higher likelihood of detecting an event and the greater number of non-detects expected with a greater sampling frequency. These effects will be controlled for by incorporating the sampling frequency into statistical analyses where possible.
- The low efficiency of recovery of *Cryptosporidium* oocysts is of concern when validating and improving the risk assessment, since there may be a high proportion of false negatives, particularly among the raw water samples where a lower efficiency filter is more likely to be used.
- Since there are so few positive samples that were suitable for species identification and many of these were mixed species or negative PCR, it is not considered feasible to use species data to stratify the risk assessment.
- The numbers of final water monitoring sites per region were too low to allow regional or seasonal stratification in analyses. However, the data suggest regional and seasonal variation which has been investigated further.

Work Package 4 - Development of the Cryptosporidium Risk Assessment Approach

Of the 228 catchment-linked WTWs, raw water sampling data were available for 73, which were used in the statistical analysis of catchment-level risk factors. 216 WTWs were validated for use in the statistical analysis of treatment performance. Spatial data that were considered potential predictor variables for *Cryptosporidium* in raw water, and for which datasets were available, were summarised for each of the 333 surface water catchments as detailed in the table below.

Dataset	Spatial resolution	Derived variables
Agricultural Census 2006/07	Parish	Animal numbers and excreta loads by catchment
Land Cover Map 2000	25m raster	Area of grassland & arable land per catchment; landuse within buffer of river network
Abattoirs and Markets	Point locations	Count per catchment
Waste Water Treatment Works	Point locations	Count per catchment
Waste Water Discharge Points	Point locations	Count per catchment
UKCIP rainfall	5km	Average annual rainfall, rainfall intensity and greatest 5-day precipitation total per catchment
InterMap Topography	5m raster	Index of 'concavity', ‘convexity’ or slope
European Soils Database	1km	Run-off index (BFI)
OS MasterMap Water Bodies	1:2,500	Bank density of surface water (lochs etc.) per catchment as index of connectivity
Countryside Survey 2000	1km	Length of field boundary that is fenced

Improved raw surface water risk assessment

Existing catchment risk assessment variables and those derived from spatial datasets that remained in multivariate models as significant predictors of *Cryptosporidium* frequency of occurrence or maximum oocyst load in raw water were the log catchment area (z=3.52, P<0.001), greatest 5-day precipitation (z=2.34, P=0.019), average slope (z=7.86, P<0.001), high numbers of birds (z.≥2.06, P≤0.017), lambing or calving (z=2.57, P=0.010), sheep excreta load (z=3.06, P<0.001), sheep pens or cattle byres (z=2.98, P=0.004), pig farms (z=2.46, P=0.016), water source type (z=4.91, P<0.001) and having no turbidity monitor on the intake (z=2.83, P=0.006). These variables were scored for entry into the revised raw water risk assessment and a catchment risk score calculated.

ROC analysis indicated that the performance of the revised catchment risk assessment is good; a large improvement on the performance on the existing RA. Logistic regression demonstrated increased odds of raw water being positive at medium risk sites (OR=5.25, 95% C.I. 1.18-23.46, P=0.030) and high risk sites (OR=33, 95% C.I. 3.48-312.6, P=0.002) compared to low risk sites. When excluding non-detects, GLM logistic regression with the number of samples positive as the outcome variable demonstrated a significantly greater frequency of occurrence of positives at medium risk (coeff=0.65, z=2.14, P=0.032) and high risk (coeff=1.40, z=4.91, P<0.001) sites compared to low risk sites, and at high risk sites compared to medium risk sites (coeff=0.75, z=6.03, P<0.001).

Improved final surface water risk assessment

The type of water treatment (P<0.001) and the catchment risk score (P<0.001) both remained in multivariate models as significant predictors of *Cryptosporidium* frequency of occurrence and maximum oocyst load in final water, along with several other variables relating to the operation of different types of filtration. A treatment risk score was calculated by summing the scores for these variables and a total score was calculated by summing the catchment and final risk scores.

ROC analysis indicated that the performance of the revised final risk assessment is good; a small improvement on the existing RA. Logistic regression demonstrated increased odds of final water being positive at medium risk sites (OR=3.93, 95% C.I. 1.89-8.20, P<0.001) and high risk sites (OR=27.53, 95% C.I. 11.33-66.93, P<0.001) compared to low risk sites. When excluding non-detects, GLM logistic regression with the number of samples positive as the outcome variable demonstrated a significantly greater proportion of positives at high risk (coeff=1.64, z=8.85, P<0.001) sites compared to low risk sites. Similarly, when excluding non-detects, the mean log (max. oocyst load x 100) increased with increasing final risk, and was significantly greater for high risk compared to low risk sites (b=0.618, t=3.97, P<0.001).

Improved ground water risk assessment

Variables from the original RA that were significant univariate predictors of *Cryptosporidium* frequency of occurrence in ground water and used in the revised risk assessment were sheep/lamb density (P=0.005), access to water source (P=0.001), rapid by-pass of unsaturated zone (P<0.001), induced recharge from surface water bodies (P<0.001), site drainage (P<0.001) and location of headworks (P=0.024). A groundwater risk score was calculated by summing the scores for these variables. ROC analysis indicated that the performance of the reduced ground water risk assessment was excellent, even though the sample size was too small to enable detection of weaker predictors.

Regional variations

Median catchment risk varied significantly between regions (Kruskal Wallis χ^2 = 37.33, P<0.001). High risk catchments appeared to be concentrated in the NE and SE, which are the regions that had highest proportion of sites with positive final water. Median final risk varied significantly between regions (Kruskal Wallis χ^2 = 9.88, P=0.011). WTWs with highest risk final waters were concentrated in the NW and the SE. WTWs in the SW had the highest proportion of low risk final waters.

Discussion points

- The statistical process described in this report provides the potential for a much improved catchment risk assessment for raw surface water that was a good predictor of *Cryptosporidium* occurrence.
- It is likely that the most 'risky' catchments (in health terms) are those that are drier on average, but have occasional high rainfall events, leading to the flushing out of a build-up of oocysts on the land.
- If the risk is 'source limited', reducing the abundance of the source (e.g. livestock) in the catchment could reduce the risk.
- A number of variables relating to the presence of farmed sheep in the catchment were associated with increased risk. Neonatal ruminant livestock have been found to be a significant reservoir of *C. parvum*, and sheep have the highest documented prevalence of oocysts in their manure.
- Pig farms and high numbers of birds in the catchment were risk factors. Pigs have particularly high concentrations of oocysts in their faeces, and birds can act as mechanical carriers and thus disseminate oocysts to surface waters.
- Removal of treatment-related variables that explained little of the variation in risk of *Cryptosporidium* occurrence in final water resulted in an improved final surface water risk assessment.
- The type of treatment was a strongly significant explanatory factor in all models.
- The presence of alarmed turbidity meters on filters, or final water, were protective, however turbidity monitoring data were not associated with the frequency of occurrence or the load of oocysts in final water. The reason for the lack of an association between turbidity and oocyst presence in Scottish water supplies warrants further investigation.
- Even though 14% of the final water positive sites were classed as low risk, these 'misclassified' sites were likely to have a lower frequency of positives and a lower oocyst load than positive sites in the other risk categories, and 'may' therefore present lower actual health risk.
- The number of ground waters managed by Scottish Water was too low for a robust statistical analysis to be performed, nevertheless several variables were strong univariate predictors of *Cryptosporidium* occurrence and their inclusion resulted in a ground water risk assessment that was an excellent predictor.
- The variables that explained most of the variation in *Cryptosporidium* occurrence in ground water were those relating to ingress of surface water.
- When investigating the ability of the treatment process to lower the final risk band into which WTWs were classified, it was apparent that applying a level of water treatment in proportion to the catchment risk was not always sufficient. Whereas, where the catchment risk was high but treatment was effective enough to remove the challenge, the risk band into which the WTW was classified could be lowered.

Recommendations

- Regular monitoring of catchment and of works operation.
- Assess seasonal variables at appropriate time of year.
- Ensure surveys of treatment performance are impartial.
- Keep a data log of plant performance.
- Use a surrogate for routine monitoring of treatment performance.
- Investigate new methods for obtaining more accurate livestock density estimates.
- Investigate higher resolution data sources for soil and climate.
- Alter cut-offs in line with resource available for monitoring if necessary.
- Increase monitoring frequency for larger populations and higher seasonal risk.

1 Background

1.1 The Cryptosporidium (Scottish Water) Directions 2003

The Scottish Government require Scottish Water to monitor drinking water supplies at a frequency determined by a risk assessment score as detailed in the *Cryptosporidium* (Scottish Water) Directions 2003 (hereafter referred to as the Directions). The number of samples that can be analysed annually for the presence of *Cryptosporidium* oocysts is limited by logistical and financial constraints. The ultimate aim of the Directions is to reduce the risk of outbreaks of *Cryptosporidiosis* due to contamination of drinking water by targeting raw and final water monitoring effort to those sites that are considered to be highest risk.

There are two independent risk assessments in the Directions – one for surface water and one for ground water. Each assesses the risk associated with (1) the catchment and (2) the treatment. The components investigated in the catchment risk assessment include the densities of farmed animals, agricultural practices, sewage inputs, water sources and intake management. The components of the treatment risk assessment include the type of water treatment, the performance of the water treatment works (WTW) and operational factors.

Each component section is scored following inspection of the catchment and the water treatment works (WTW), and overall scores are calculated for the catchment and for the treatment by summing section scores. The catchment risk score, along with the WTW design flow, is used to inform the *raw* water monitoring frequency (Table 1). A risk assessment score for the *final* water supply is obtained by summing the catchment and treatment scores and is used, along with the WTW design flow, to determine the final water monitoring frequency (Table 2).

Table 1 Raw water annual monitoring frequency specified in the 2003 Directions

		WTW Maximum Design Flow (Ml/day)			
		≤ 1	**>1 ≤ 10**	**>10 ≤ 50**	**> 50**
Catchment Risk Score	**> 55**	12	26	52	52
	35 – 54	0	12	12	26
	< 35	0	0	12	12

Table 2 Final water annual monitoring frequency specified in the 2003 Directions

		WTW Maximum Design Flow (Ml/day)			
		≤ 1	**>1 ≤ 10**	**>10 ≤ 50**	**> 50**
Catchment + Treatment Risk Score	**> 55**	52	104	365	365
	35 – 54	12	52	52	104
	< 35	12	12	52	52

The final risk assessment score is weighted by 0.4 x the log of the population served by the supply, and this final weighted score is used to assign the supply to a high, medium or low risk category. If the water supply is considered high risk (final weighted score >100), the sampling frequency requirement may be greater than that in Table 1 & Table 2, dependent upon the community prevalence of cryptosporidiosis.

All WTW sites have final water monitoring in place at a minimum frequency of once a month (Table 2). Sites may not be required to monitor their raw water if the combination of catchment risk score and design flow is low enough (Table 1), or if there is no treatment except disinfection, hence no difference in the raw and final water (Rasool *et al*., 2004).

1.2 Cryptosporidium sampling

Samples are taken at the frequency determined by the Directions, with a minimum flow rate through each sampling unit of 40 litres per hour for a minimum period of 24 hours and a maximum period of 36 hours (where less than 365 samples are required in a one year period). Scottish Water have two types of filters for use as sampling units for *Cryptosporidium*- Genera filters and Cuno filters. Genera filters are used on all final water supplies as they have a better recovery rate (30-60%) than Cuno filters (≤20%) (Rasool *et al*., 2004). Cuno filters have to be used on poorer quality raw water (high turbidity) for technical reasons.

It is required that final (i.e. treated potable) water samples are analysed at an appropriate laboratory within three days of sample acquisition, and that raw waters are analysed within five days. Continuous sampling is triggered by an event in a catchment that may significantly increase the possibility of *Cryptosporidium* oocysts entering the raw water supply, or if there is any deviation in turbidity as measured by turbidity meters at the WTW. Such reactive samples should be analysed within 36 hours of sampling. A sample is defined as ‘positive’ where the oocyst count is >0 per 10 litres, otherwise the sample is classed as a ‘no detect’ rather than a negative, due to the low recovery efficiency of the filters.

Species identification can only be performed if a sample contains two or more oocysts (pers. comm. Prof. H. Smith), which excludes many of the *Cryptosporidium* positive samples. Where samples have been speciated, *Cryptosporidium spp.* found have included *C. parvum, C. hominis, C. andersoni, C. bovis*, *Cryptosporidium* muskrat genotype and cervine genotypes. Often there are mixed species within a sample.

2 Work Package 2 - Data Collation and Validation

2.1 Cryptosporidium monitoring data 2006-07

2.1.1 Samples

For the purposes of this project, *Cryptosporidium* monitoring data for the 22-month period January 2006 to October 2007 were available, and all statistics presented here relate to that time period. A total 24,205 samples were collected, 3,776 (16%) of which were raw water samples and 20,249 (84%) final water samples. Of the raw water samples, 965 (26%) were positive for Cryptosporidium, with a maximum oocyst count of 7.3 $10L^{-1}$. Of the final water samples, 1484 (7%) were positive, with a maximum oocyst count of 16.2 $10L^{-1}$. The higher maximum oocyst count in final water compared to raw water may be as a result of the lower recovery efficiency from raw water due to the type of filter.

2.1.2 Water Sources

A total of 304 final waters were monitored during the 22 month study period, 266 (87.5%) of which were sourced from surface water and 38 (12.5%) from groundwater. Only 80 raw waters were monitored for *Cryptosporidium*, all of these being surface waters. Of these, 65 (81%) were positive at some point, and of the final waters sampled, 165 (54%) were positive. For positive waters only, the distribution of the proportion of raw water samples positive per WTW is shown in Figure 1, and the distribution of the proportion of final water samples positive per WTW, stratified by water source type (surface or ground), is shown in Figure 2.

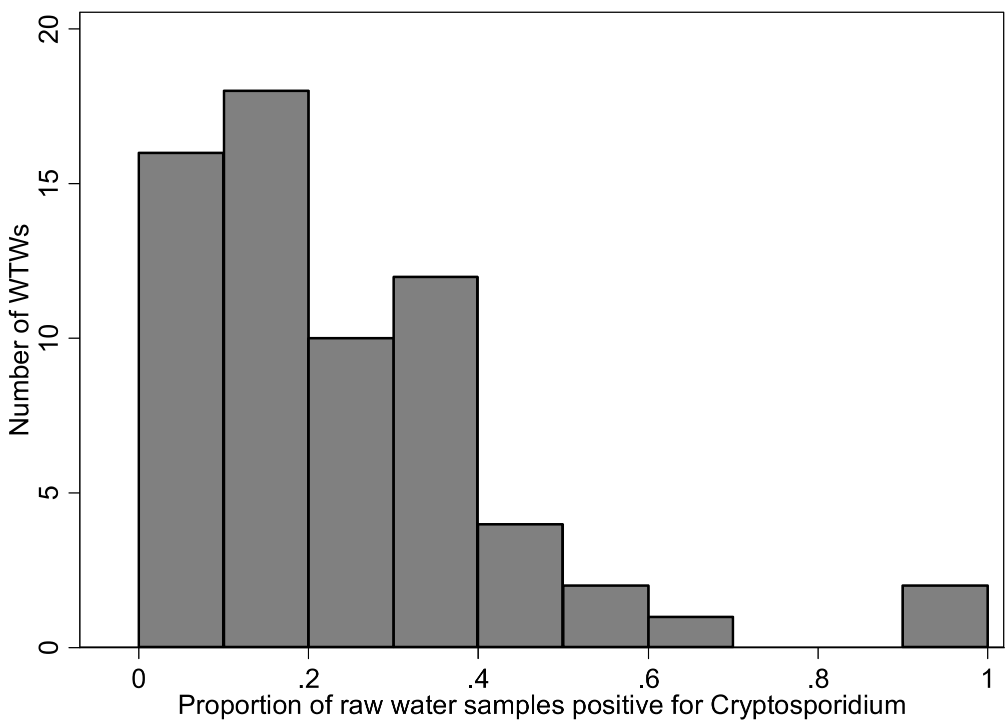


Figure 1. Histogram of the proportion of raw water samples per WTW that were positive for *Cryptosporidium*. Data is shown for raw waters that had at least one positive sample.

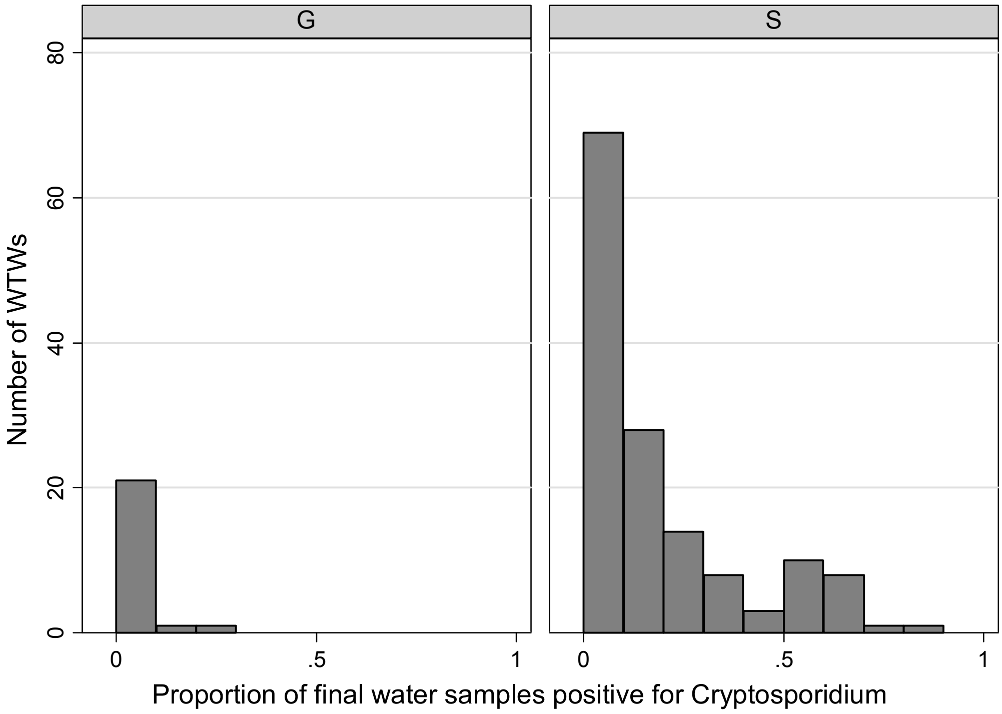


Figure 2. Histogram of the proportion of final water samples per WTW positive for *Cryptosporidium*, stratified by water source type (G=groundwater; S=surface water). Data is shown for final waters that had at least one positive sample.

The distribution of the maximum oocyst count per WTW where *Cryptosporidium* was detected in raw water is shown in Figure 3, and the distribution of the maximum count in final water at positive WTWs, stratified by source type (surface or ground) is shown in Figure 4.

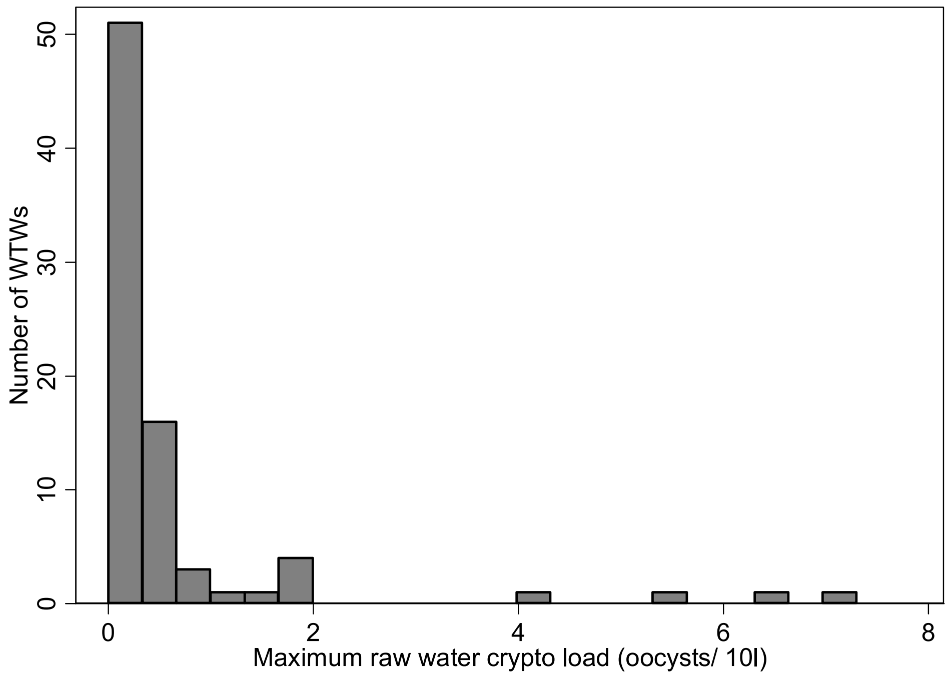


Figure 3. Histogram of the maximum oocyst load per WTW in positive raw water samples.

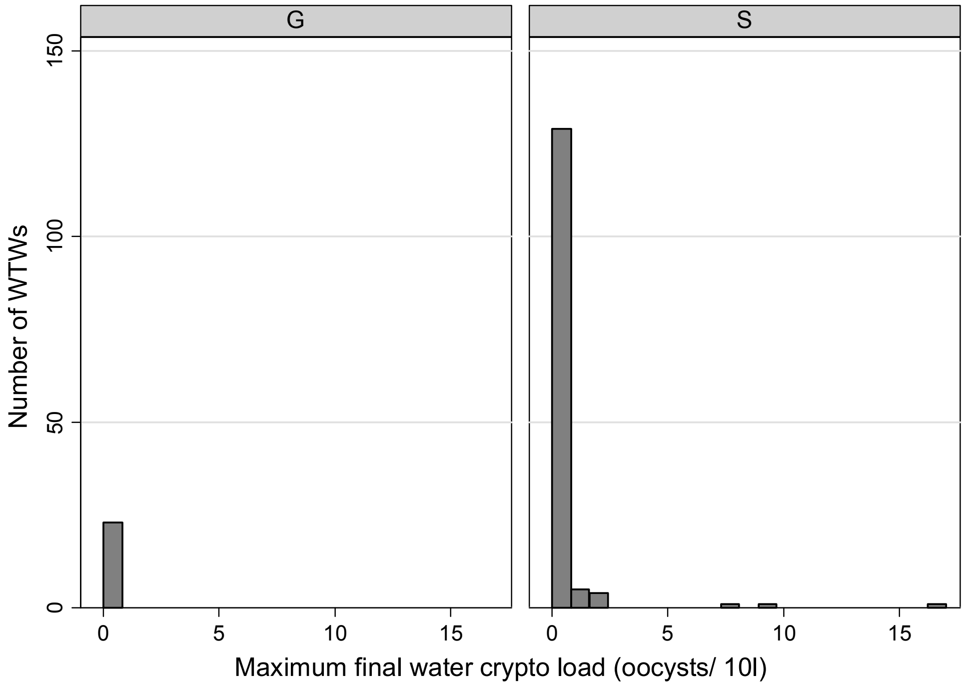


Figure 4. Histogram of the maximum oocyst load per WTW in positive final water samples, stratified by water source type (G=groundwater; S=surface water).

2.1.3 Regional differences in surface water *Cryptosporidium*

The numbers of ***surface*** water sites with raw and final water sampling in each operational region along with the percentage of these that were positive for *Cryptosporidium* during the study period are shown in Table 3. There was no statistically significant difference in the proportion of surface water sites that had positive raw water between regions (Pearson's χ^2= 2.92, *P*=0.40), but there was a statistically significant difference in the proportion of surface water sites that had positive *final* water between regions (Pearson's χ^2= 15.65, *P*=0.001), with the South East having the highest and the South West the lowest (Figure 5). In a strict statistical sense, this might be taken to suggest that the probability of *Cryptosporidium* entering raw waters in a catchment does not differ regionally, but the performance of the water treatment to remove *Cryptosporidium* does; however the sample size for raw water was much smaller than that for final water. This could be interpreted in relation to past outbreak patterns, with Glasgow getting more attention to treatment.

Table 3. Numbers of surface water WTWs with raw and final water sampling for *Cryptosporidium* and the percentage of these that were positive during 2006-07, stratified by operational region.

Region	**Raw water**		**Final water**	
	# sites sampled	**# (%) sites positive**	**# sites sampled**	**# (%) sites positive**
NE	18	16(89)	23	16(70)
NW	16	13(81)	158	87(55)
SE	15	10(67)	31	23(74)
SW	31	26(84)	48	16(33)

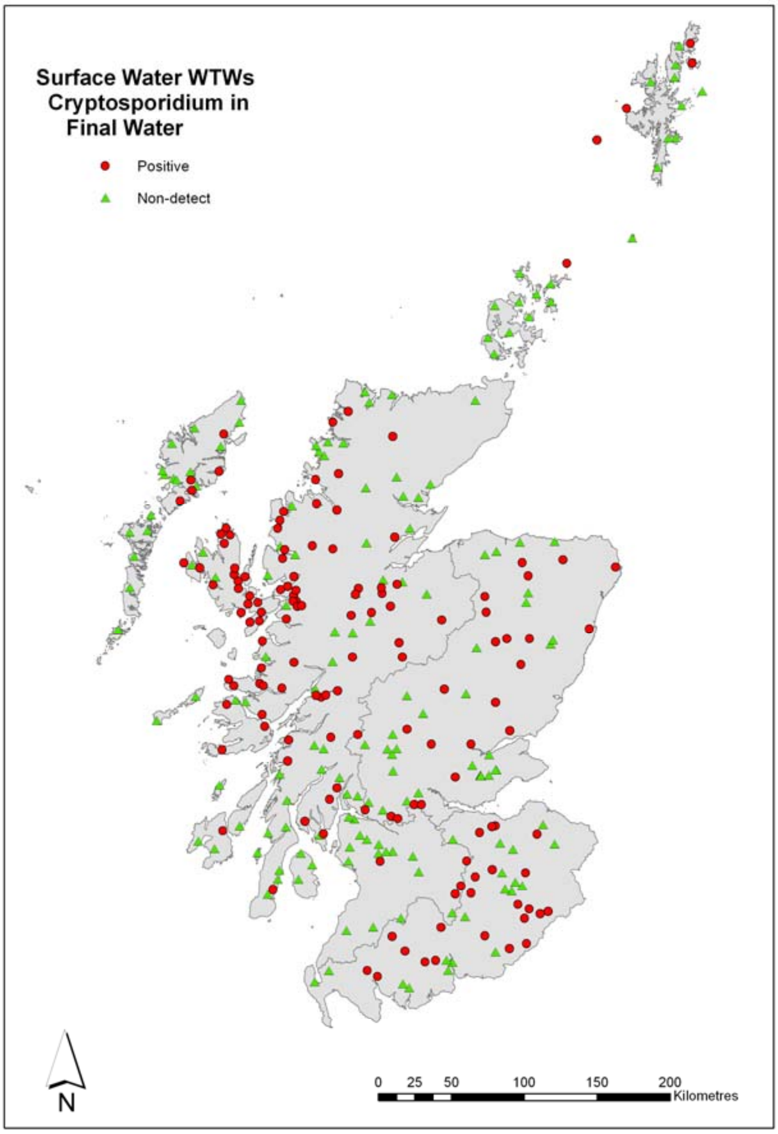


Figure 5. Locations of WTWs within the operational regions in Scotland symbolised according to whether or not they had a positive *Cryptosporidium* final water sample in 2006-07.

The numbers of raw and final surface water samples taken per region, plus the percentage of these that were *Cryptosporidium* positive are shown in Table 4. The proportion of samples positive per WTW did not differ significantly by region for raw water samples (Kruskal-Wallis χ^2= 5.95, P=0.114), but there were significant regional differences for final water samples (Kruskall-Wallis χ^2= 14.51, P=0.002), with the North West having the highest proportion of positives and the North East the lowest (Figure 6).

Table 4. Numbers of raw and final surface water samples taken per operational region and the percentage of these that were positive for *Cryptosporidium*.

Region	Raw water		Final water	
	# samples	**#(%) samples positive**	**# samples**	**#(%) samples positive**
NE	1149	313(27)	3593	124(3)
NW	362	42(12)	5079	880(17)
SE	797	265(33)	2477	225(9)
SW	1451	344(24)	3639	141(4)

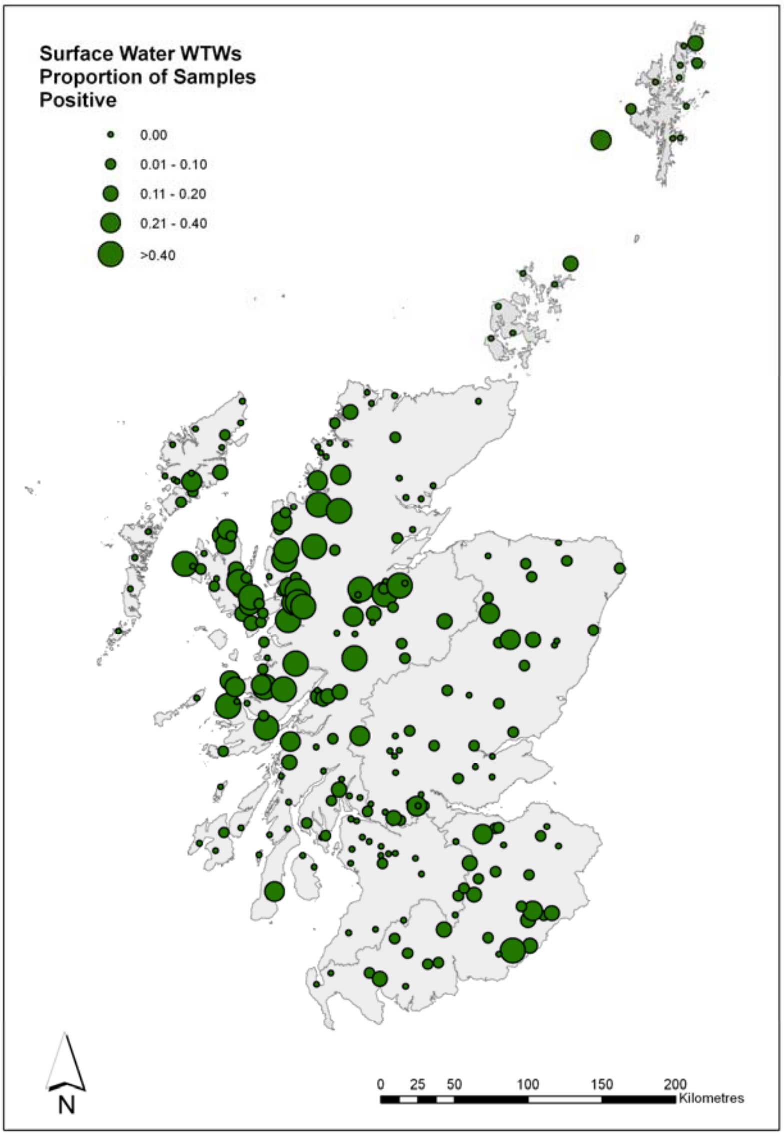


Figure 6. Surface water WTWs symbolised according to the proportion of their final water samples that were positive for *Cryptosporidium* from 2006-07.

2.1.4 Species data

Species data were available for 1054 positive samples from 2005-06. Of these, 325 were from 107 WTWs sampled in 2006. These 325 samples had sufficient oocyst counts to attempt species identification using a combination of PCR, RFLP and sequencing, 62 (19%) were identified as *C. andersoni*, 26 (8%) were a cervine genotype, 17 (5%) were *C. parvum*, 1 (0.3%) was *C. baileyi*, 1 (0.3%) was *C. bovis*, 1 (0.3%) was *C. muris*, 1 (0.3%) was a muskrat genotype, 26 (8%) were an unidentifiable *Cryptosporidium* species, 137 (42%) had a negative PCR result, 26 (8%) were yet to be sequenced, and the remaining 27 (8%) were mixtures of species. The most prevalent species detected (*C. andersoni*) is thought to be mostly host specific to bovines (Xiao *et al*., 2004).

2.1.5 Seasonality

There is evidence of some seasonality in the final water sampling data (Figure 7), with a peak occurring in November/December and troughs in April and June. We may expect a rainfall association and an autumnal flush effect (pers. comm. Prof. David Kay), and indeed there is a significant correlation between *Cryptosporidium* prevalence in final water samples and average monthly rainfall (Spearman's *rho*=0.76, *P*=0.005) (Figure 7). The seasonality of the data will be investigated further and will be considered when testing the effects of seasonal risk factors such as winter rainfall and lambing.

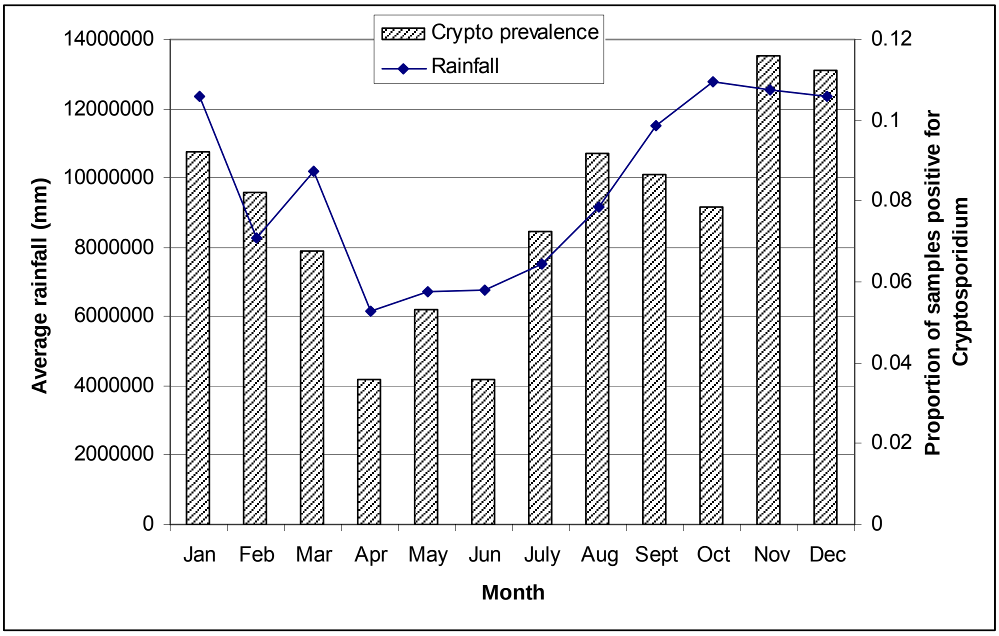


Figure 7. The proportion of final water samples positive for *Cryptosporidium* by sample month, and the average monthly rainfall.

2.2 Risk Assessment scores 2006-07

2.2.1 Overall scores

The median catchment risk score of the 266 surface water sources was 44 (range 22-90), compared to 84 (range 26-137) for the 38 groundwater sources. There was a significant difference between the distributions of the catchment scores for surface water and groundwater (K-W χ^2= 51.85, *P*<0.001) (Figure 8). The median overall risk scores before population weighting were 34 (range -5-96) and 83 (range 33-150) for surface and groundwaters respectively. There was a significant difference in overall risk score distribution between surface water and groundwater sites (K-W χ^2= 73.14, *P*<0.001) (Figure 9).

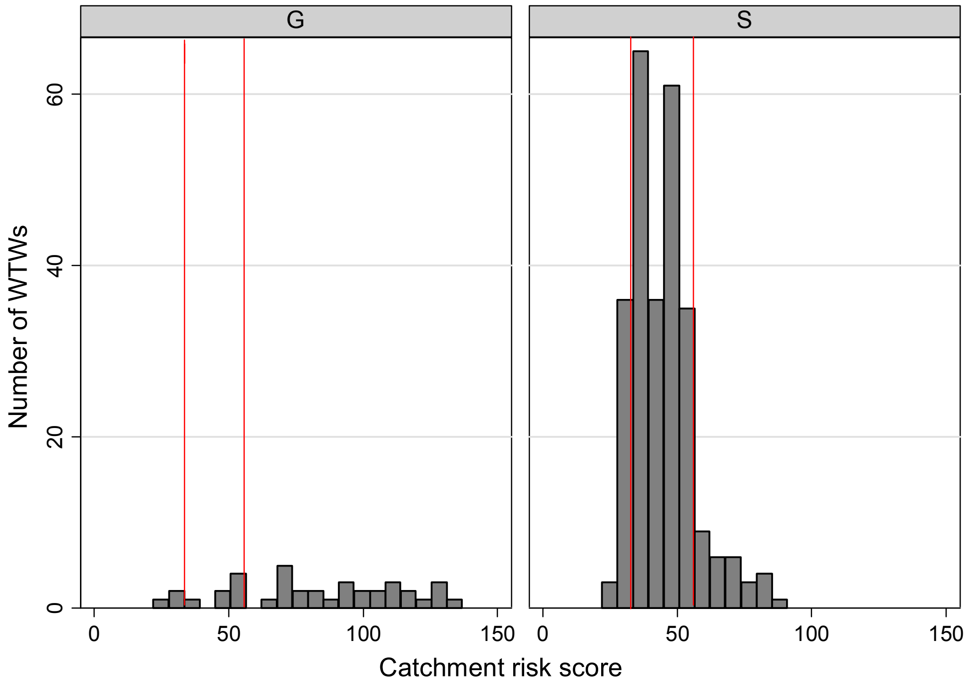


Figure 8. Histogram of the catchment risk scores per WTW, stratified by water source type (G=groundwater; S=surface water). Red lines indicate the approximate cut-off points in the Directions for determining sampling frequency of raw water.

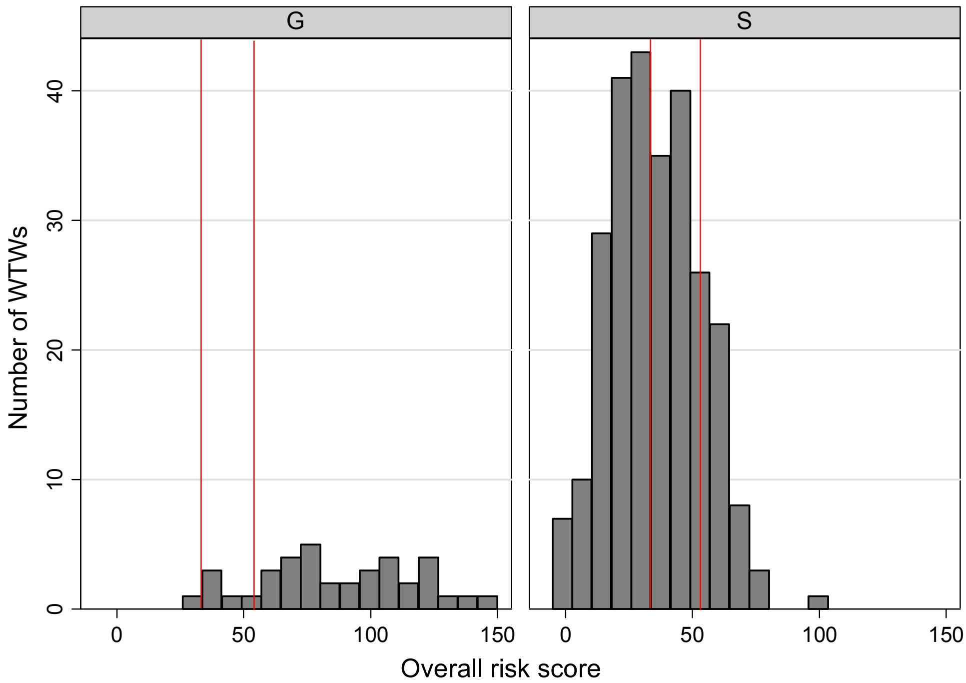


Figure 9. Histogram of the overall unweighted risk scores per WTW, stratified by water source type (G=groundwater; S=surface water). Red lines indicate the approximate cut-off points in the Directions for determining sampling frequency of final water.

The final water sampling schedule for sites in 2007 is shown in Figure 10.

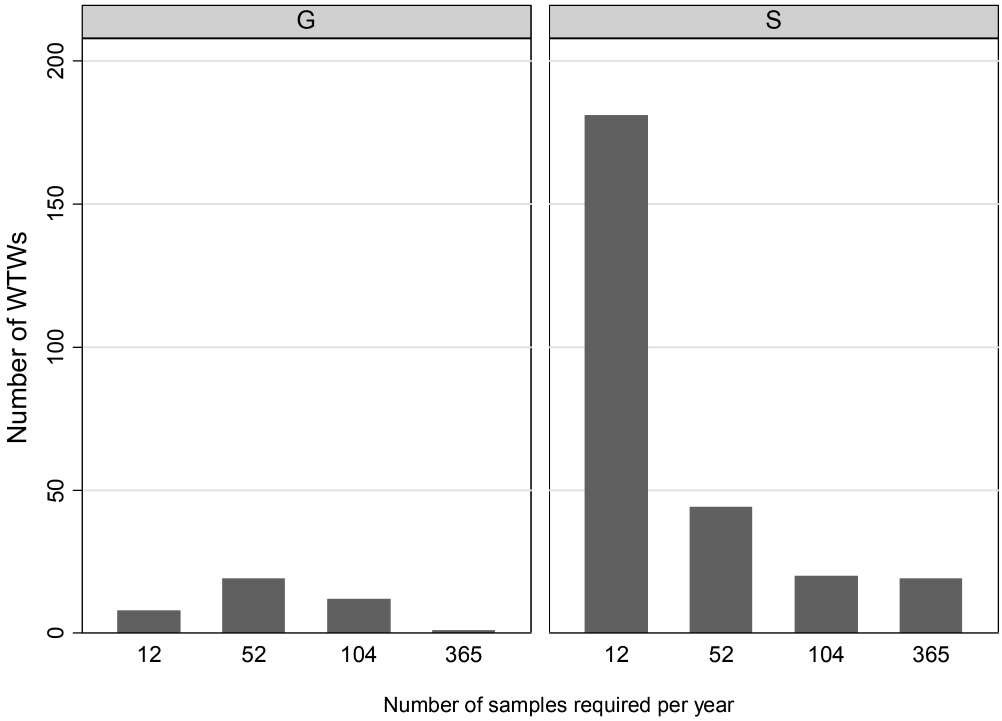


Figure 10. The numbers of final water samples required for WTWs in 2007, stratified by the water source type (G=groundwater; S=surface water).

Of the 304 WTWs with risk assessment scores, there were 17 whose final weighted score changed between 2006 and 2007, mainly due to alteration of treatment process, or changes in the population served by the supply.

2.2.2 Individual factor scores

The variables within each section of the surface water and groundwater risk assessments are shown in Appendix 1, along with the distribution of observations between the outcomes of each potential risk factor and the score associated with each.

2.2.3 Regional differences in surface water risk assessment scores

The median surface water catchment and overall unweighted surface water risk assessment scores by region are shown in Table 5. The median catchment risk assessment score varied significantly between regions (Kruskal-Wallis χ^2= 27.28, P<0.001) (Figure 11), with the North East having the highest and the North West the lowest. The median final unweighted surface water risk assessment score also varied significantly between regions (Kruskal-Wallis χ^2= 31.94, P<0.001) (Figure 12), with the North East having the highest and the South West the lowest. Again, this may reflect improved water treatment in the South West region.

Table 5. Median surface water catchment and overall risk assessment scores by operational region.

Region	Catchment score		Overall score	
	median	**95% C.I.**	**median**	**95% C.I.**
NE	57	44-77	45	31-54
NW	41	39-44	38	33-42
SE	47	37-53	40	22-45
SW	45	42-45	23	18-26

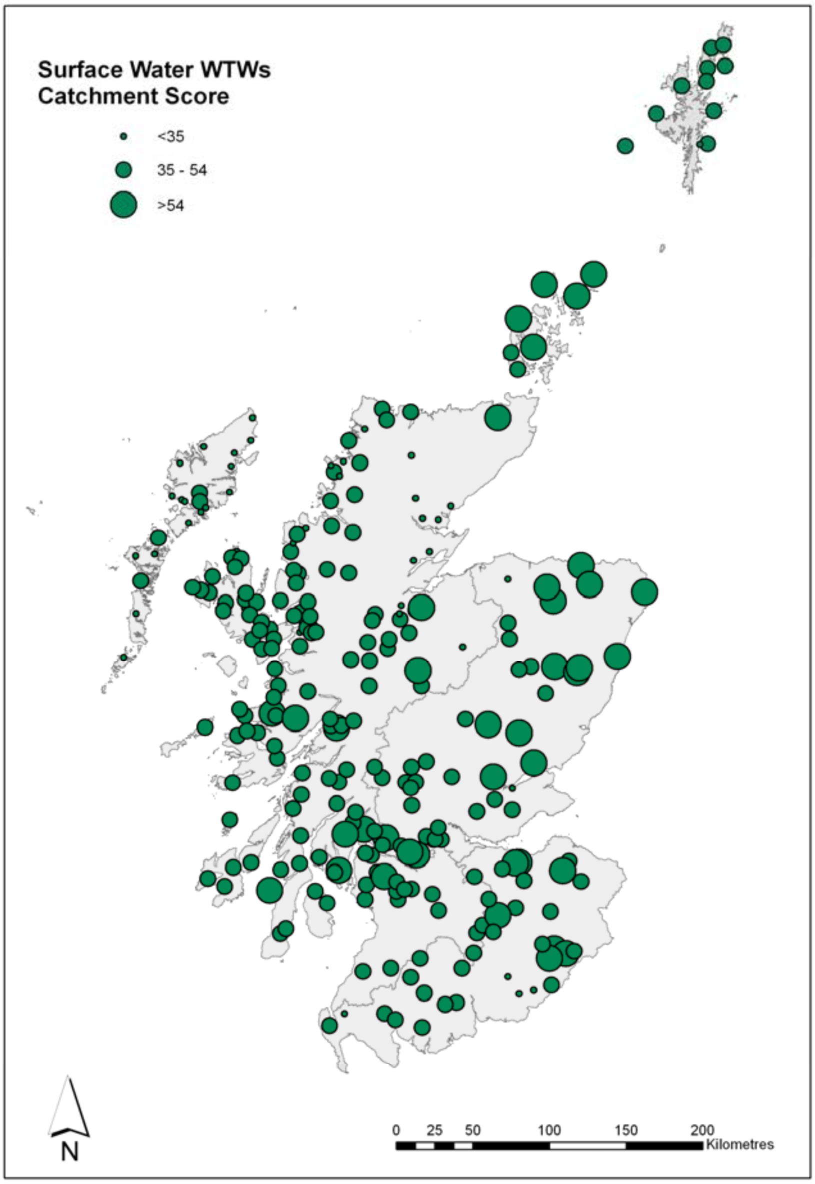


Figure 11. Distribution of surface water catchment risk assessment scores between operational regions. Cut-offs are the same as those used in the Directions.

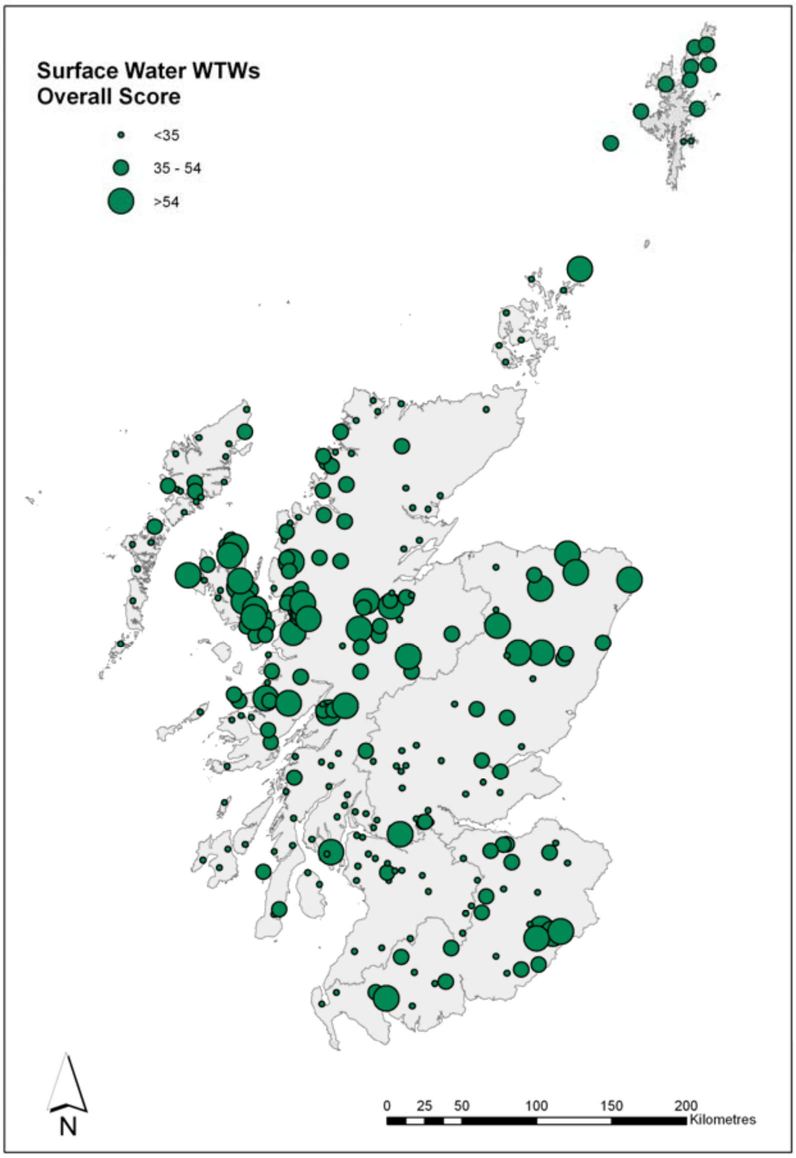


Figure 12. Distribution of overall unweighted surface water risk assessment scores between operational regions. Cut-offs are the same as those used in the Directions.

2.3 Hydrological catchments and abstraction points

Catchment boundaries were provided for 702 hydrological catchments, 412 of which are for Scottish Water source waters. Of these, 381 are surface water catchments. The distribution of surface water catchment sizes was highly skewed, having a median of 350ha (95% C.I. 277-407), and a range of 3-510,784 ha. Scottish Water has a total of 534 abstraction points to supply the 304 WTWs, and the numbers of these raw water sources that are reservoirs, boreholes & springs, rivers & burns and lochs is shown in Figure 13.

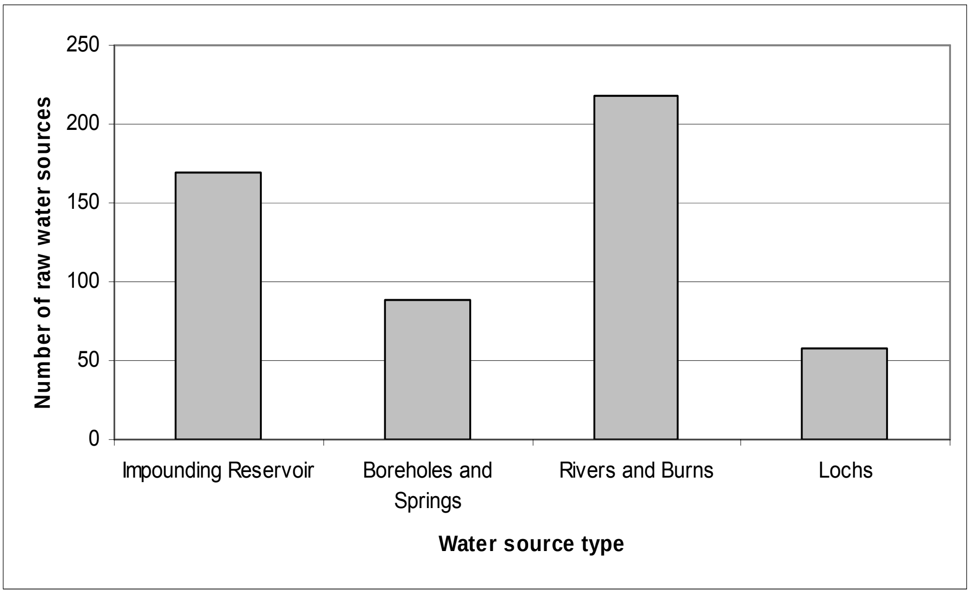


Figure 13. The distribution of raw water sources between the four main types in Scotland.

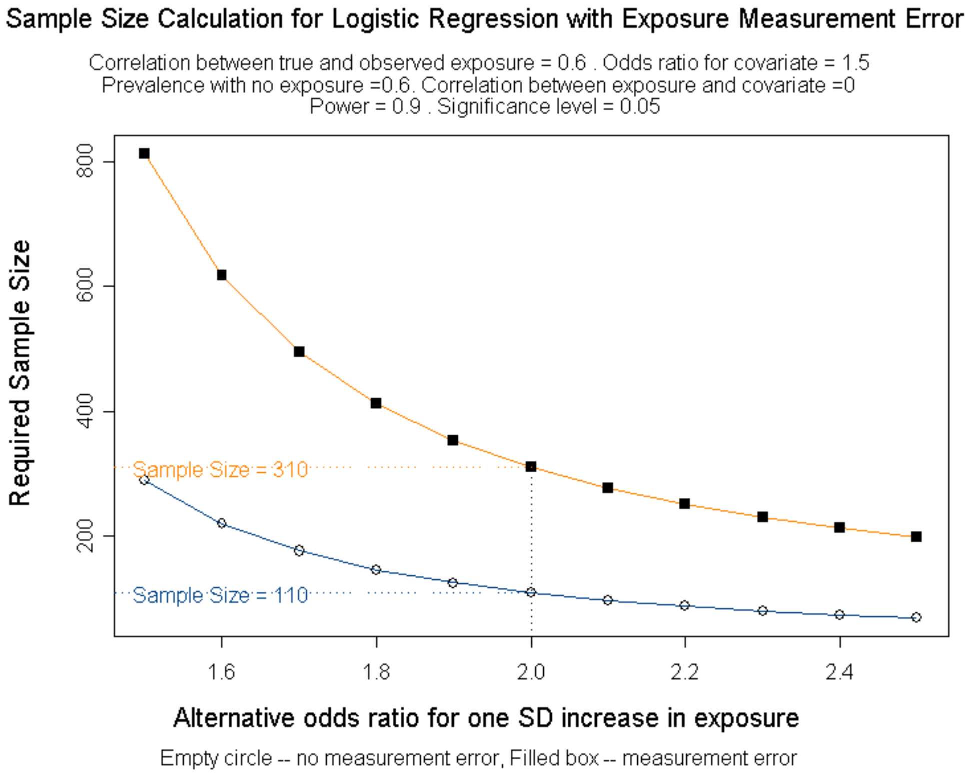
2.4 Turbidity monitoring data

There are a total of 16066 turbidity monitoring records for the 22 month study period, 142 (0.9%) of which failed the PCV for turbidity (1 Nephelometric Turbidity Unit (NTU)).

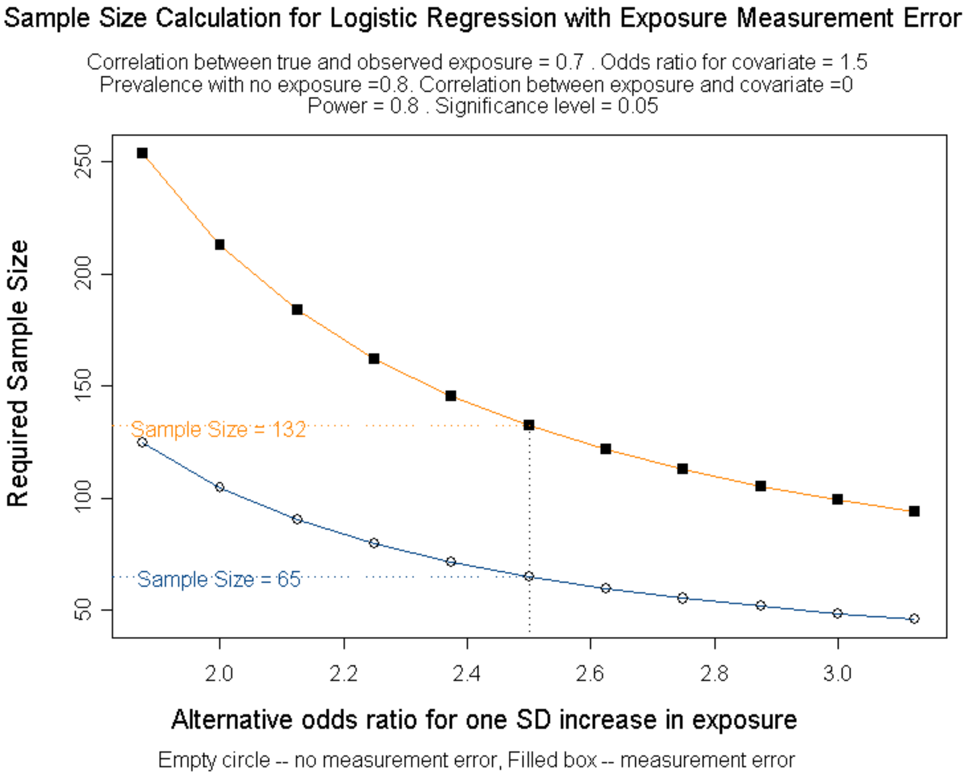
3 Work Package 3 – Evaluation of the *Cryptosporidium* Risk Assessment Approach

3.1 Statistical Power

There are a total of 266 surface waters with risk assessment data and final water monitoring for *Cryptosporidium*. Of these, 80 also have raw water monitoring data. There are 38 groundwater sources with risk assessment data and final water monitoring data. A program that provides sample size or power in logistic regression (Tosteson *et al*., 2003) was used to estimate the power of the available sample sizes to detect a difference in *Cryptosporidium* outcome. To detect a 2-fold change in *Cryptosporidium* outcome (binary) with 90% power at a significance level of 0.05 for one standard deviation increase in the exposure variable, a sample size of between 110 and 310 is required depending on whether or not a normally distributed measurement error model is incorporated for the exposure variable (Figure 14a). This calculation is based on the prevalence of *Cryptosporidium* occurrence (binary) in final water at the mean for the risk assessment score. To detect an odds ratio of 2.5 with 80% power at a significance level of 0.05, a sample size of between 65 and 132 would be needed, depending on whether or not measurement error is accounted for (Figure 14b). This calculation is based on the prevalence of the binary outcome in raw water at the mean for the risk assessment score.



(a)



(b)

Figure 14. Sample size calculations for logistic regression with (a) 90% power to detect an odds ratio of 2.0 and (b) 80% power to detect an odds ratio of 2.5.

These calculations show that the sample size for final surface waters (n=266) will be sufficient to detect a 2-fold increase in risk of *Cryptosporidium* in the final water with 90% power (assuming a low measurement error for the exposure variable), but the sample size for groundwaters (n=38) is insufficient assuming the same criteria. For this reason, the performance of the groundwater risk assessment cannot be statistically validated. The sample size for raw surface waters (n=80) is sufficient to detect a 2.5-fold increase in risk of *Cryptosporidium* in the raw water with 80% power (given a low measurement error for the exposure variable).

3.2 The performance of the existing risk assessment

3.2.1 Raw Surface Water

Of the 80 sites with raw water monitoring data, 2 had changes in their risk assessment score in 2006/07 and were therefore excluded from analyses. This gave a sample size of 78 on which to assess the performance of the surface water catchment score to predict *Cryptosporidium* occurrence in the catchment. A total of 28 raw waters were considered high risk, of which 25 (89%) had at least one positive sample in 2006/07 (Table 6). Only 5 raw waters that were classed as low risk were sampled, of which 3 (60%) had at least one positive sample in 2006/07. Sampling of low risk raw waters is less common since the WTW maximum design flow has to be >10 ML/day for routine sampling to be required for low risk catchments as per the Directions (see Table 1).

Table 6. Summary table showing the distribution of monitored raw waters that had/ did not have *Cryptosporidium* detections in the study period 2006/07 between catchment risk categories.

Catchment risk score	Non-detects	Positives	Total
>55	3 (20%)	25 (40%)	28
35-54	10 (67%)	35 (55%)	45
<35	2 (13%)	3 (5%)	5
Total	15 (100%)	63 (100%)	78

Receiver operating characteristic (ROC) analysis shows that the maximum concurrent sensitivity and specificity of the catchment risk category to predict *Cryptosporidium* occurrence in raw water only reaches about 60% (Figure 15). The accuracy of the 'test' is measured by the area under the ROC curve, which in this case is 0.62 (95% C.I. 0.48-0.76). An area of 1 represents a perfect test, whereas an area of 0.5 represents a worthless test. An area of 0.62 indicates that the test is poor, based on the traditional academic points system which provides a rough guide for classifying the accuracy of a diagnostic test: 0.9-1.0 is considered an excellent test, 0.8-0.9 is good, 0.7-0.8 is fair, 0.6-0.7 is poor and 0.5-0.6 is a fail.

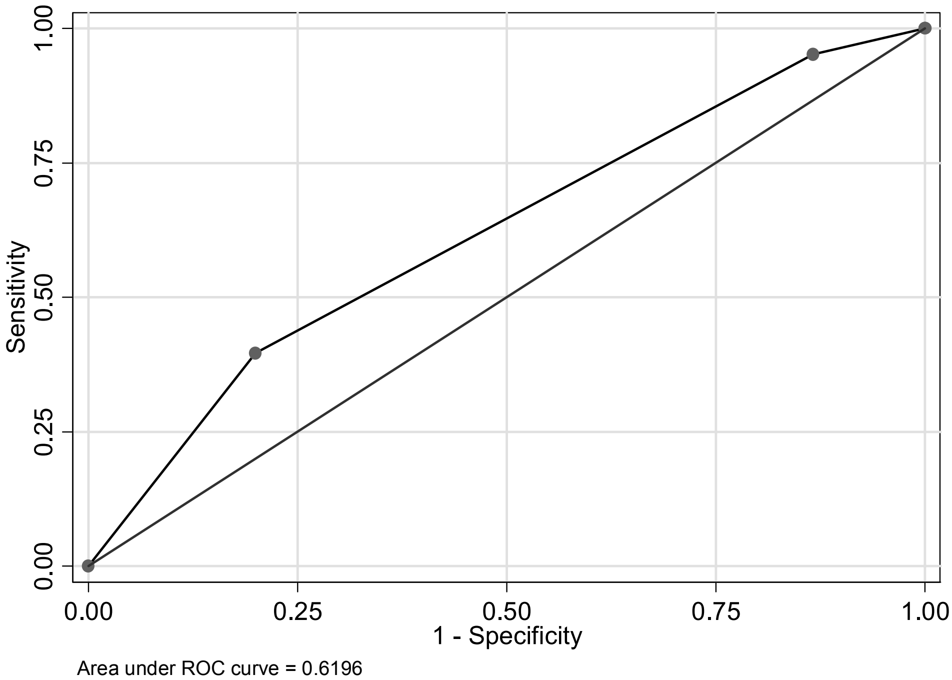


Figure 15. ROC curve showing the accuracy of the raw water risk assessment score to predict *Cryptosporidium* occurrence in the catchment.

Using logistic regression, there was shown to be an increased odds of positive raw water if the catchment was scored as medium risk (OR=2.33, 95% C.I. 0.34-15.95) or high risk (OR=5.56, 95% C.I. 0.65-47.84) compared to low risk, but these increased odds were not significant, as evident by the large confidence intervals, which is likely due to the low number of observations in the low risk category and/or measurement error in the risk assessment scores.

Using a generalized linear model with a binomial distribution and a logit link, with the number of samples positive as the outcome variable and the number of samples taken as the denominator, raw waters that were scored as medium risk had a significantly greater proportion of samples positive than low risk sites (b=2.16, z=4.20, P<0.001), as did those that were scored as high risk (b=1.95, z=3.80, P<0.001). High risk sites actually had a lower proportion of samples positive than medium risk sites (b=-0.21, z=-2.47, P=0.013). These results suggest that using the proportion of samples positive may be a more accurate representation of risk than a binary outcome, possibly because of the lack of variation in a binary outcome (there is a high likelihood of oocysts being present in raw water at some point over the study period), and also because sampling frequency is taken into account in this regression model.

3.2.2 Final Surface Water

Of the 266 surface water sites with final water monitoring data, 16 had inconsistent risk assessment scores for 2006/07 and a further 5 were missing *Cryptosporidium* monitoring data and were thus excluded from analyses. This gave a sample size of 245 on which to assess the performance of the final surface water score to predict *Cryptosporidium* occurrence in the final water. A total of 38 final waters were considered high risk, of which 37 (97%) had at least one occurrence of *Cryptosporidium* in 2006/07 (Table 7). The majority of final waters (50%) were considered low risk, of which 82 (67%) had no occurrences of *Cryptosporidium* in 2006/07.

Table 7. Summary table showing the distribution of monitored final waters that had/ did not have *Cryptosporidium* detections in the study period 2006/07 between overall unweighted risk categories.

Overall risk score	Non-detects	Positives	Total
>55	1 (1%)	37 (27%)	38
35-54	24 (22%)	61 (44%)	85
<35	82 (77%)	40 (29%)	122
Total	107 (100%)	138 (100%)	245

ROC analysis shows that the maximum concurrent sensitivity and specificity of the overall unweighted risk category to predict *Cryptosporidium* occurrence in final surface water reaches approximately 75% (Figure 16). The area under the ROC curve is 0.77 (95% C.I. 0.71-0.82), indicating that the performance of the test is fair according to the academic points system. We could infer that the level of treatment is of greater importance than catchment level risk factors, judging by the better performance of the final risk assessment to correctly identify positive sites compared to the catchment risk assessment.

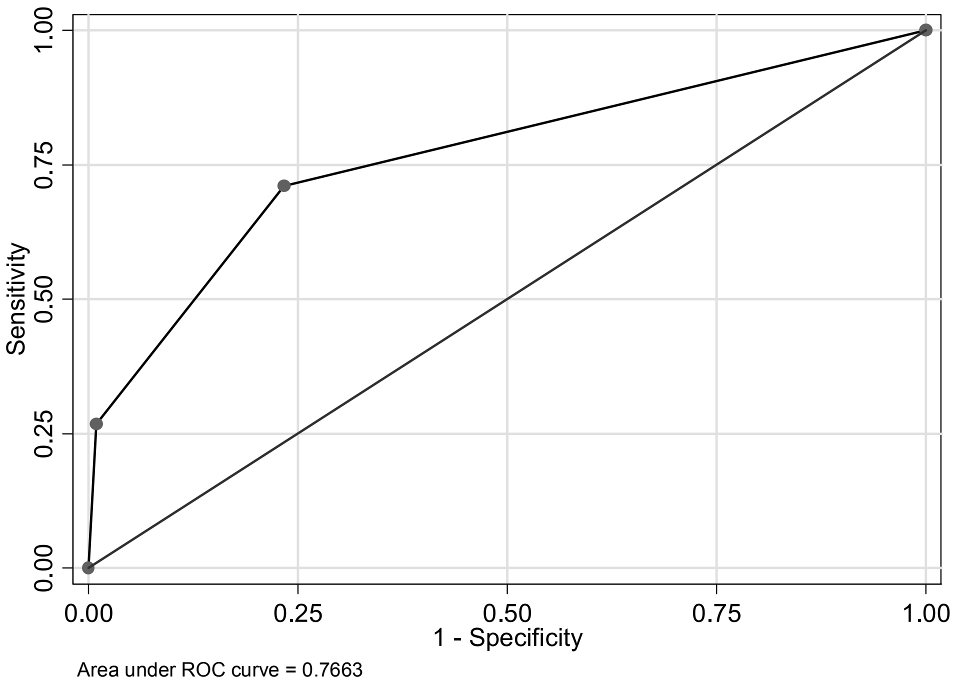


Figure 16. ROC curve showing the accuracy of the overall surface water risk assessment score to predict *Cryptosporidium* occurrence in the final water.

Logistic regression demonstrated increased odds of final surface being positive medium risk sites (OR=5.21, 95% C.I. 2.85-9.54, $P<0.001$) and high risk sites (OR=75.85, 95% C.I. 10.04-572.77, $P<0.001$) compared to low risk sites. High risk sites also had a significantly higher likelihood of *Cryptosporidium* in final water when compared to medium risk sites (OR=14.56, 95% C.I. 1.89-112.12, $P=0.010$). The high odds ratios reported here may reflect a higher probability of a positive due to a higher sampling frequency (see section 4.3.).

Using a generalized linear model with a binomial distribution and a logit link, with the number of samples positive as the outcome variable and the number of samples taken as the denominator, final surface waters that were scored as medium risk had a significantly greater proportion of samples positive than low risk sites (b=2.11, z=17.36, $P<0.001$), as did those that were scored as high risk (b=2.52, z=21.55, $P<0.001$). High risk sites had a higher proportion of samples positive than medium risk sites (b=0.42, z=6.41, $P<0.001$).

3.3 Limitations of the Cryptosporidium monitoring data

3.3.1 Raw water monitoring data

A large proportion (73%) of WTWs did not have raw water monitoring data for this period, which limited the validation and improvement of the catchment risk assessment score. The useable sample size of 78 monitored raw waters is marginal in terms of having sufficient statistical power to assess the performance of the catchment risk assessment, but only five of these fall within the low risk category, which causes further issues for statistical validation. To mitigate this problem, catchments whose final water is positive for *Cryptosporidium* will be included in alternate analyses to boost the sample size, since these would have also had positive raw water samples, but this may bias the sample to catchments with high starting loads in the raw water and/or poor treatment performance. One way of controlling for this will be to include a binary signal variable in the regression model to reduce the effect of the final water sites. It will not be feasible to include catchments in which no *Cryptosporidium* was detected in the final water, since no assumptions can be made as to the status of the raw water as treatment may, or may not, have removed any oocysts that were present.

3.3.2 Assessment of treatment process

To assess the performance of the treatment process it will only be valid to use data from sites that have positive raw water samples, since the effectiveness of the treatment can only be tested if challenged by oocysts in the raw water entering the treatment works. To improve on the treatment score in the risk assessment, it would have been preferable to temporally link raw water positives to final water positives. The effectiveness of the treatment could then be measured by the log reduction in oocyst load attained by the treatment process. In order to do this, the time that elapses between water entering and leaving a WTW must be known. This time is not standard between WTWs and is an unknown for each WTW, making this analysis approach unachievable. An alternative will be to use the improved catchment risk score as a surrogate for the *Cryptosporidium* load in the raw water entering the WTW, but this will only be possible if the catchment risk score is a good predictor of *Cryptosporidium* load.

3.3.3 Sampling frequency

There are two issues to be addressed with regards sampling frequency. Firstly, the likelihood of detecting a contamination event will increase with sampling frequency, such that sites that are designated as high risk, and therefore on a higher frequency sampling schedule, will by design have a higher chance of being a positive site over the course of a year or two. The challenge here is to separate the effects of the sampling schedule from the true predictive power of the risk assessment.

Secondly, the proportion of samples that are *Cryptosporidium* positive will be dependent upon the frequency of sampling, both due to a higher likelihood of detecting an event and the greater number of non-detects expected with a greater sampling frequency. These effects may cancel each other out to a certain extent, but in any case, sampling frequency should be accounted for in statistical analyses, either by incorporating sampling frequency as a weighting, or including it as an exposure variable. This can be done when analysing the outcome as a binomial proportion using GLM, but is more difficult when assessing the performance of the risk assessment using a binary outcome, since the frequency of sampling is determined by the risk assessment score and, thus, cannot be included as a confounder.

3.3.4 Recovery efficiency

The efficiency of recovery of *Cryptosporidium* oocysts is of concern when validating and improving the risk assessment, since there may be a high proportion of false negatives, particularly among the raw water samples as a lower efficiency filter is more likely to be used for raw water. This will also have implications for measurements of log reduction in oocyst count from raw to final water, since the count in raw water may be an underestimate compared to that in final water. It is difficult to control for such uncertainties in analyses, as data on the efficiency of recovery of oocysts is not available at a site level. It may be possible to obtain an estimate of the false negative rate for raw water samples by examining time series monitoring data for sites that have positive final water samples but non-detects in raw water. Otherwise, the assumption must be made that the likelihood of recovery of oocysts is higher if the starting load is higher, and thus positive samples represent the highest risk water sources.

3.3.5 Regional variation

The number of final water monitoring sites per region were too low, with the exception of the NW (see Table 3), to allow regional stratification in analyses. However, the data suggest regional variation which will be investigated further.

3.3.6 Species data

Since there are so few positive samples that were suitable for species identification and many of these were mixed species or negative PCR, it is not considered feasible to use species data to stratify the risk assessment.

4 Work Package 4 – Development of the *Cryptosporidium* Risk Assessment Approach

4.1 Linking Hydrological catchments with abstraction points

Scottish Water supplied catchment boundaries for 713 raw waters, of which 412 could be linked to a raw water source using a Raw Source Site Reference to Raw Catchment ID lookup (Figure 17). Of these, 381 (92%) were surface water catchments. Thirty-six of the surface water abstraction points were linked to between two and seven catchments. The locations of these 36 abstraction points in relation to their linked catchment boundaries were inspected in a GIS and the most appropriate hydrological catchment for the abstraction point chosen based on the location of surface waters overlaid onto a DTM. The resulting catchment dataset comprised 333 surface water catchments that could be linked to 333 abstraction points.

4.2 Linking WTWs to catchments

Of the 259 surface WTWs with risk assessment data and final water monitoring, 228 (88%) could be linked to a Raw Catchment via their Raw Source(s) lookup (Figure 17). Of these, 174 sourced their raw water from one catchment each and 54 sourced their water supply from between two and eight catchments. For the WTWs that sourced their raw water from >1 catchment, catchment level variables were either summed, or if this was not appropriate, a worst case taken. Of the 228 catchment-linked WTWs, raw water sampling data were available for 73, which were used in the statistical analysis of catchment-level risk factors. Twelve of the WTWs had inconsistent risk assessment data between 2006 and 2007, therefore the remaining 216 WTWs were used in the statistical analysis of treatment performance.

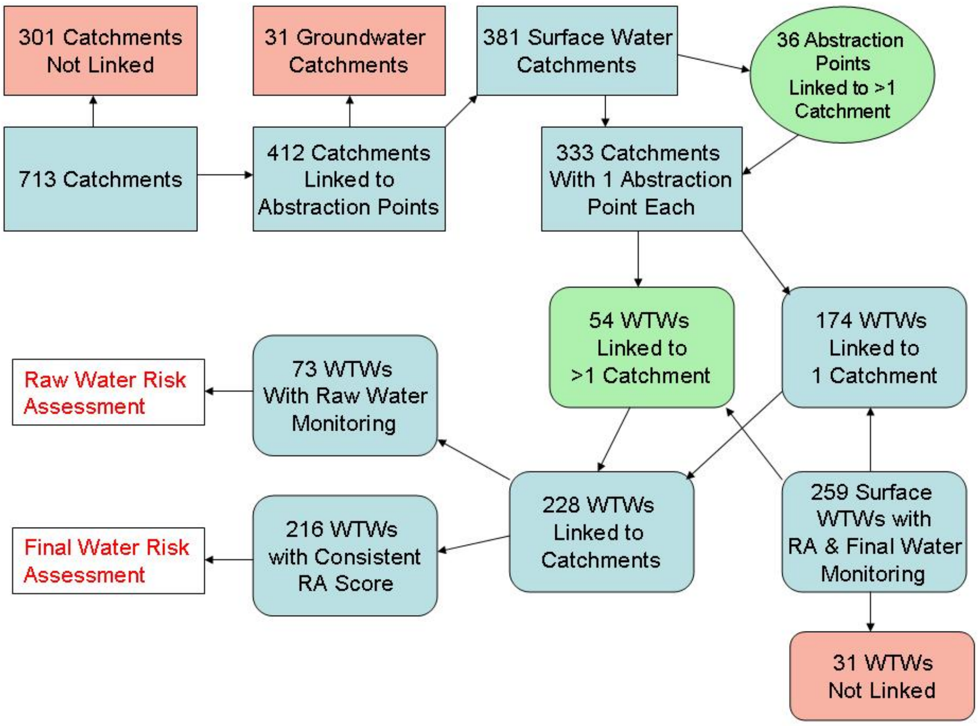


Figure 17. Schematic showing the selection of WTWs for use in statistical analyses of risk assessment variables.

4.3 Catchment-level spatial data

Spatial data that were considered potential predictor variables for *Cryptosporidium* in raw water, and for which datasets were available (Table 8), were summarised for each of the 333 surface water catchments. Descriptions of the datasets and the methodology for extracting catchment-level data from each are detailed below.

Table 8. **Available spatial datasets, their spatial resolution and proposed use as catchment level explanatory variables**

Dataset	Spatial resolution	Derived variables
Agricultural Census 2006/07	Parish	Animal numbers and excreta loads by catchment
Land Cover Map 2000	25m raster	Area of grassland & arable land per catchment; landuse within buffer of river network
Abattoirs and Markets	Point locations	Count per catchment
Waste Water Treatment Works	Point locations	Count per catchment
Waste Water Discharge Points	Point locations	Count per catchment
UKCIP rainfall	5km	Average annual rainfall, rainfall intensity and greatest 5-day precipitation total per catchment
InterMap Topography	5m raster	Index of 'concavity', 'convexity' or slope
European Soils Database	1km	Run-off index (BFI)
OS MasterMap Water Bodies	1:2,500	Bank density of surface water (lochs etc.) per catchment as index of connectivity
Countryside Survey 2000	1km	Length of field boundary that is fenced

4.3.1 Land Cover

Land Cover Map 2000 (LCM2000 - CEH) is a 25m raster dataset. Each of the grid cells records the dominant land cover at that location in terms of LCM2000 Subclasses. LCM2000 Subclasses were combined to obtain estimates of the area of grassland, arable land and rough grazing within each catchment. Grassland was taken as the 'Improved grassland' subclass (5.1). The estimate of arable land area summed subclasses 4.1, 4.2, 4.3 & 5.2, which are 'Arable cereals', 'Arable horticulture', 'Arable non-rotational' and 'Setaside grass'. The rough grazing category was calculated by summing subclasses 6.1, 7.1, 8.1, 9.1, 10.1, 10.2 & 11.1, which are 'Neutral grass', 'Calcareous grass', 'Acid grassland', 'Bracken', 'Dense dwarf shrub heath', 'Open dwarf shrub heath' and 'Fen, marsh, swamp'.

4.3.2 Agricultural Census

Parish-level agricultural census data from June 2007 along with the parish boundaries were provided by the Scottish Government. Numbers of Dairy cattle, Beef cattle, Other cattle, Calves, Sheep, Lambs, Pigs and Poultry were summed per parish. The area of grassland, arable land and rough grazing were summarized per parish from LCM2000. Livestock numbers were used in conjunction with the land cover data to obtain estimates of the stocking density of each of the livestock categories per unit area of forage. For Dairy cattle, the forage area was taken to be grassland only. For Beef cattle, Other cattle, Calves, Sheep and Lambs, the forage area was taken to be the combined grassland and rough grazing areas.

For Pigs and Poultry, grassland plus arable land was used as the areal denominator. A total livestock density was calculated using grassland plus rough grazing as the forage area.

Catchment polygons that overlaid more than one parish were intersected with the parish boundaries to create a set of sub-catchment polygons. Land cover data was calculated per sub-catchment as in the previous section. Each sub-catchment polygon was then updated with the stocking density of each of the livestock categories in its coincident parish. These stocking densities were multiplied by the appropriate land cover area to obtain estimates of numbers of livestock in each of the categories per sub-catchment, and summed by original catchment ID. Stocking densities per catchment were calculated by dividing the livestock numbers by the appropriate area of forage.

4.3.3 Manure Management Database

ADAS' Manure Management Database (MMDB) is a spatially and temporally distributed database of manure applications across a range of agricultural land uses, and was constructed to facilitate a range of modelling activities associated with manure management and loadings on agricultural land of manure-borne pathogens for example. It integrates national and regional manure practice survey data with local agricultural census data. The survey practice data are used as weights against the local data on crop areas and animal numbers, from which total excreta production are calculated. The MMDB was used to obtain estimates of the quantity of excreta (i.e. directly voided) produced for each livestock category per catchment. Calves and lambs were considered separately from adult cattle and sheep due to the expected higher load of *Cryptosporidium* oocysts in the excreta of younger animals (Hutchison *et al*., 2002).

4.3.4 Land use within a buffer of water bodies

The OS Master Map (OSMM) Rivers dataset contains polyline data for all lower order streams and rivers (captured at 1:2,500 scale, or 1:10,000 in rural areas), and outlines the edges of larger rivers and other water bodies. The polyline data was intersected with the catchment polygons and a 50m buffer was created around the outside edges of lochs and rivers. The areas of grassland and arable land within the buffer zones were summarised per catchment using LCM2000, and the proportion of the buffer area that comprised grassland and arable land calculated.

4.3.5 Connectivity

An estimate of river and loch bank density per catchment was obtained from OSMM Water Bodies and OSMM Rivers datasets. Both datasets were intersected with the catchment boundaries. The perimeter length of the polygon features and the length of the polyline features within each catchment were calculated. The perimeter length was subtracted from the line length (to avoid double counting) and the remaining line length was multiplied by two to obtain an estimate of bank length either side of the polyline rivers. The two values were then summed to give an overall bank length for the catchment, and divided by the area of the catchment to obtain a bank density.

4.3.6 Point Sources

The point locations of Abattoirs & Markets, Waste Water Discharges and Waste Water Treatment Works for Scotland were provided by the Scottish Government. The numbers of each of these potential point sources of *Cryptosporidium* were summarised per catchment.

4.3.7 Soil Data

The dominant Soil Typological Unit (STU) for each 1x1km grid cell in Scotland was obtained from the European Soils Database (ESDB) v.2. Each STU can be associated with a particular Hydrology of Soil Types (HOST) class, which describes dominant pathways of water movement through soil. HOST can be used to predict the Base Flow Index (BFI) of a soil (Schneider *et al*., 2007), which is a good indicator of the runoff potential, and is used here as a proxy of the susceptibility of catchments to surface water contamination with *Cryptosporidium* oocysts (Leu *et al,* 2008). 1km grid cells that intersected the catchment boundaries were selected, and the dominant HOST class within each catchment calculated.

4.3.8 Rainfall

Annual long-term averages (1961-90) for rainfall (mm), greatest five-day precipitation total (mm) and rainfall intensity (annual total of wet days (>1mm rain) / number of days giving a wet day rainfall) are available from UKCIP at 5km grid cell resolution. Means of each of these parameters were calculated per catchment from the intersecting grid-cells.

4.3.9 Linear Features

Linear feature survey data was downloaded from the Countryside Information System (CIS), which gives a crude estimate of the total linear feature length (hedges, walls, fences etc.) within each 1km grid cell based on the Institute of Terrestrial Ecology's land class mean. These values were then summed per catchment using area weighted statistics of the underlying grid cells.

4.3.10 Topography

Estimates of convexity and concavity were calculated for each catchment from 5m DTMs (InterMap), but the variation between catchments was too small for this to be a useful indicator of run-off risk. An alternative simple indicator of the steepness of the catchment and thus the run-off risk was the mean percentage slope, which varied much more between catchments and was thus used as a risk assessment variable.

4.4 Statistical Analyses

Two measures of *Cryptosporidium* in both raw and final waters were used as outcome variables in comparative models in order to maximise the likelihood of identifying risk or protective factors for the risk assessment. The first outcome variable used was the number of sampling occasions on which *Cryptosporidium* was detected, and was analysed using a generalized linear model (GLM) with a binomial distribution and logit link, which enabled the denominator (the number of samples taken) to be incorporated into the equation. The second outcome variable was the maximum oocyst load (oocysts/100L) multiplied by 100 and logged, which normalised the data so it could be analysed using ordinary least squares (OLS) regression. By considering the risk factors identified using both of these outcome variables, the probability of missing significant factors due to the way in which *Cryptosporidium* risk was estimated (i.e. as a frequency of occurrence or a maximum load) was reduced.

4.4.1 Raw Water Risk Assessment

Categorical or binary variables were generated using the current RA scores, and were each tested univariately and then entered into multivariate models if significant at $P \leq 0.05$. Alteration of the weights of these variables did not affect their predictive power. Variables were removed from the multivariate model by backward stepwise elimination of non-significant variables that, when removed, caused a decrease in Akaike's Information Criteria (AIC). A minimum AIC strategy can be used for selecting among two or more competing models – generally, the model for which AIC is the smallest represents the 'best' approximation to the true model (Dayton, 2003).

Catchment level variables that had been extracted from available datasets (4.3) were tested univariately as potential risk factors for *Cryptosporidium* prevalence or load in raw water. If the continuous variable was significant at $P \leq 0.05$, percentiles were used to split the variable into three categories, and the categorical variable was re-tested. If the intermediate category did not differ significantly from the lower, the low and intermediate groupings were combined to generate a binary variable.

On obtaining reduced models using only existing RA variables, catchment-derived variables that were significant univariate predictors were added in turn, and retained in the final models (one for each outcome variable) if they remained significant and resulted in a decrease in AIC. Scores were assigned to categories of any extra catchment-derived variables that remained in either of the final models based on their contribution towards explaining the variation in the outcome. Weights were restricted to the scoring range of the existing variables. A catchment score was calculated for each WTW by summing the scores for each variable that remained in either of the final models. Cut-off scores to assign a WTW to a catchment risk band (low, medium or high) were initially based on percentiles of the catchment score to obtain approximately equal numbers in each category. The power of the catchment risk score to predict *Cryptosporidium* occurrence in raw water was tested using logistic regression and ROC analysis, and the area under the ROC curve compared to that of the original RA. The cut-offs and scores were adjusted to maximise the area under the ROC curve. The differences in the frequency of occurrence of *Cryptosporidium* and the mean log maximum oocyst load in raw water between risk categories were statistically evaluated using GLM and OLS regression respectively.

4.4.2 Final Water Risk Assessment

All of the WTWs with consistent RA data and final water monitoring data that could be linked to a catchment were used in the analysis of the predictive power of the treatment RA variables. Since raw water monitoring data were only available for 73 of the 216 eligible WTWs and considering the difficulty with temporally linking raw water samples to final water samples, the new raw water risk category was entered into the statistical models as a covariate to act as a surrogate measure of *Cryptosporidium* frequency of occurrence or load in raw water entering the WTW. This allowed the performance of the final water RA variables to be assessed whilst controlling for the *Cryptosporidium* risk in the raw water. Since many of the factors in the final water RA are specific to a certain type of treatment, the categorical treatment variable was also entered as a covariate when testing the other variables. This enabled additional scores relating to the operation of a filtration system to be assessed in combination with the treatment score.

Existing treatment RA variables were tested univariately as risk or protective factors for *Cryptosporidium* prevalence or load in final water. Variables were tested using their existing scores, and for all WTWs where a variable was not scored in the RA (i.e. the question was not applicable to the type of filtration), it was given a score of 0 so that all observations were used in the analyses. All variables that remained significant at $P \leq 0.05$ were entered into multivariate models. Models that gave the best fit were obtained by backward stepwise elimination of non-significant variables that, when removed, caused a decrease in AIC. A variable for the turbidity of the final water (low, medium, high) was tested for its potential as a predictor alongside the RA variables in the reduced model. A treatment score was calculated for each WTW by summing the scores for each variable that remained in either of the final models. The treatment score was added to the catchment score to give a final score for each WTW. Cut-off scores to assign a WTW to a final risk band (low, medium or high) were based on percentiles of the final score to obtain approximately equal numbers in each category as a starting point, and cut-offs shifted until the area under the ROC curve was maximised.

The power of the final risk score to predict *Cryptosporidium* occurrence in final water was tested using logistic regression and ROC analysis, and the area under the ROC curve compared to that of the original RA. The differences in the frequency of occurrence of *Cryptosporidium* and the mean log maximum oocyst load in final water between risk categories were statistically evaluated using GLM and OLS regression respectively.

4.4.3 Groundwater Risk Assessment

As concluded in WP3, there are too few groundwater sources to have confidence in any statistical analyses of the data unless very strong associations are evident, and groundwater catchments are too small to derive any spatial parameters with a sufficient degree of accuracy. However, the existing groundwater RA data were assessed for their predictive power to give an indication of which variables *might* be most important in determining sampling frequency for groundwater sources. First, groundwater sources with RA data (N=38) were spilt into two groups based on whether or not they had had a *Cryptosporidium* PCV failure during the study period. The distribution of the RA scoring between these groups was then investigated, which gave an indication of which variables may be important in predicting PCV failures in groundwater. The variables that were potential predictors were tested in univariate regression models (GLM), with the number of samples that failed the PCV as the outcome variable, with the number of sampling occasions as a denominator. Maximum oocyst load did not vary sufficiently to be used as an outcome variable. Variables that were significant predictors ($P \leq 0.05$) in univariate analyses were entered into a multivariate model, and the model that gave the best fit was obtained by backward stepwise elimination of non-significant variables that, when removed, caused a decrease in AIC. Scores for the variables that remained in the model were summed per WTW, and assigned a risk band based on the distribution of final scores. The predictive power of the risk category was tested using ROC analysis and the area under the curve compared to that obtained when including all variables that were significant at the univariate stage in the risk score.

4.5 *Results*

4.5.1 Raw Water Risk Assessment

The catchment-level variables that were derived from spatial datasets are shown in Table 9, along with their means, standard deviations and ranges for the analysis sample (N=216).

Table 9. Catchment-level variables derived from spatial datasets along with descriptive statistics for the sample used in analyses (N=216).

Variable	Mean	Std. Dev.	Min	Max
Land Area Variables				
Area of catchment (ha)	1954	4978	0.21	42095
Proportion of catchment improved grassland	0.044	0.108	0	0.86
Proportion of catchment arable land	0.006	0.036	0	0.45
Proportion of 50m surface water buffer improved grassland	0.092	0.153	0	0.913
Proportion of 50m surface water buffer arable land	0.016	0.050	0	0.375
River/lake bank density (m/ha)	59.0	21.7	17.3	124.1
Linear Feature Density (m/ha)	33.8	21.6	4.4	98.3
Livestock Variables				
Dairy cattle density (ha^{-1})	0.074	0.184	0	1.06
Beef Cattle Density (ha^{-1})	0.083	0.121	0	0.70
Other Cattle Density (ha^{-1})	0.049	0.110	0	1.08
Calf Density (ha^{-1})	0.074	0.110	0	0.54
Sheep Density (ha^{-1})	0.771	0.595	0.05	2.83
Lamb Density (ha^{-1})	0.663	0.611	0.02	3.16
Pig Density (ha^{-1})	0.035	0.291	0	4.12
Poultry Density (ha^{-1})	1.513	8.062	0	78.33
All Livestock Density (ha^{-1})	1.803	1.907	0.09	15.06
Dairy Excreta Load (kg/ha)	3.13	7.73	0	44.32
Beef Excreta Load (kg/ha)	2.61	3.79	0	21.74
Other Cattle Excreta Load (kg/ha)	1.46	3.31	0	32.27
Calf Excreta Load (kg/ha)	0.73	1.11	0	5.38
Sheep Excreta Load (kg/ha)	3.16	2.44	0.19	11.62
Lamb Excreta Load (kg/ha)	0.73	0.67	0.03	3.48
Pig Excreta Load (kg/ha)	0.14	1.12	0	15.91
Poultry Excreta Load (kg/ha)	0.13	0.69	0	6.74
Hydrological/ Topographical Variables				
Base Flow Index	0.23	NA	0.23	0.9
Average Annual Rainfall (mm)	1862	570	837	3577
Rain Intensity (mm/rain day)	8.22	1.83	4.05	13.93
Greatest 5-day Precipitation (mm)	107	30	43	225
Average Slope (%)	9.19	5.34	0.86	27.64
Point Sources				
Abattoirs and Markets	0.005	NA	0	1
Waste Water Treatment Works	0.088	NA	0	11
Waste Water Discharge Points	0.083	NA	0	10

Many of the variables varied substantially between catchments, in particular the land area variables. The proportion of the catchment that was managed agricultural land was generally very low, although the data were highly skewed, with a large number of essentially non-agricultural catchments and a small number that were >20% agricultural. Consequently, mean livestock densities and related excreta loads were low, but again the data were highly skewed. Hydrological/ topographical variables varied significantly for them to be tested as explanatory variables. None of the catchments contained an abattoir or market from the point locations dataset, with the exception of one catchment, which had a market. Seven catchments contained ≥1 WWTW, of which 4 had ≥1 waste water discharge point.

4.5.1.1 Performance of existing RA variables in univariate analyses

The existing catchment risk assessment variables that were significant univariate predictors of *Cryptosporidium* frequency of occurrence or load (tested as categorical variables without weights) are shown in Table 10. Catchments that had a high number of birds, sheep pens or cattle byres and lambing or calving had both a higher frequency of occurrence and maximum load than did catchments without these factors. Water source type (scored as in the existing RA) was a significant univariate predictor of both frequency of occurrence and load, as were whether or not intakes were shut under poor water quality and whether or not there was a turbidity monitor on the intake.

Table 10. Existing catchment RA variables that were significant univariate predictors of either of the *Cryptosporidium* outcomes along with their regression coefficients and significance level.

Existing RA Variable	Frequency of Occurrence		Log Maximum Load	
	GLM Coeff.	*P*	OLS Coeff.	*P*
Deer in catchment	0.296	0.001		
High numbers of birds	0.268	<0.001	0.551	0.002
Sheep pens or cattle byres	0.093	<0.001	0.712	<0.001
Lambing or calving in catchment	0.153	<0.001	0.689	0.009
Pig farms in catchment			1.654	0.028
Dung or slurry storage			0.528	0.048
Abattoir/ livestock market			1.654	0.028
Septic tanks for population >100	0.449	0.004		
Storm water outlets	0.268	0.034		
Water Source Type	0.047	0.051	0.128	0.013
Intakes not shut under poor WQ	2.430	<0.001	0.480	0.002
No turbidity monitor in intake	1.846	<0.001	0.388	0.009

4.5.1.2 Performance of additional catchment level variables in univariate analyses

The additional catchment level variables detailed in Table 9 that were significant univariate predictors of *Cryptosporidium* frequency of occurrence or load are shown in Table 11, along with their categorical classifications for potential entry into the improved RA. Larger catchments (>7 ha) had both a higher frequency of occurrence and a higher load than smaller catchments (≤7 ha), and a higher BFI (>0.42 cf. ≤0.42) was also associated with a higher frequency of occurrence and higher load. Annual average rainfall had a positive association with the frequency of occurrence, but a negative association with load, which may be indicative of the dilution effect of rainfall on oocysts in raw water.

Table 11. Additional catchment level variables that were significant univariate predictors of either of the *Cryptosporidium* outcomes along with their regression coefficients and significance level.

Potential RA Variable	Category	Frequency of Occurrence		Log Maximum Load	
		GLM Coeff.	***P***	**OLS Coeff.**	***P***
Log Catchment Area (ha)	0-7				
	>7-8	1.339	<0.001	0.374	0.079
	>8	1.285	<0.001	0.549	0.013
Proportion Grassland	0				
	>0-0.05	0.787	0.001		
	>0.05	0.190	0.423		
Proportion Arable	0				
	>0-0.01	-0.069	0.446		
	>0.01	-0.318	0.006		
Bank Density (m/ha)	0-45				
	>45-60	0.545	<0.001		
	>60	0.904	<0.001		
Linear Feature Density (m/ha)	0-30				
	>30-50	-0.256	0.006		
	>50	-1.030	<0.001		
Base Flow Index	0-0.26				
	>0.26-0.42	-0.333	0.108	0.558	0.050
	>0.42	0.456	<0.001	0.438	0.016
Annual Average Rainfall (mm)	0-1300				
	>1300-1800	0.797	<0.001	-0.320	0.133
	>1800	0.557	<0.001	-0.503	0.019
Rainfall Intensity (mm/rain day)	0-7				
	>7-9	0.696	<0.001		
	>9	0.601	<0.001		
Greatest 5-day Precipitation (mm)	0-90				
	>90-100	0.007	0.961		
	>100	0.723	<0.001		
Average Slope (%)	0-6				
	>6-10	0.142	0.368		
	>10	1.216	<0.001		
Waste Water Treatment Works (p/a)		0.525	<0.001		
Sheep Excreta load (kg/ha)				0.095	0.030
Lamb Excreta load (kg/ha)				0.303	0.050
All Livestock density (ha^{-1})				0.102	0.008

Univariate analyses of catchment level variables were also performed on an expanded dataset (N=161), which included WTWs that had at least one positive final water sample, and including a binary flag for the sample type (raw or final) as a covariate. Coefficients and significance levels were very similar to those from the raw water dataset only (results not shown), therefore the raw water dataset (N=73) was used in multivariate analysis.

4.5.1.3 Multivariate models

The final minimal adequate models including both existing and potential RA variables that best predicted *Cryptosporidium* frequency of occurrence or load in the raw water are shown in Table 12 and Table 13.

Table 12. Variables that remained in the minimal adequate model to explain the variation in the frequency of occurrence of *Cryptosporidium* in raw water.

Variable	Coefficient	z[1]	*P*
Log Catchment Area >7	0.765	3.52	<0.001
Greatest 5-day Precipitation >90mm	0.366	2.34	0.019
Average Slope >10%	0.868	7.86	<0.001
High numbers of birds	0.128	2.38	0.017
Lambing or calving in catchment	0.095	2.57	0.010
Water Source Type	0.164	4.91	<0.001

[1] The test statistic **z** is the ratio of the Coefficient to the Standard Error of the respective predictor. The **z** value follows a standard normal distribution.

Table 13. Variables that remained in the minimal adequate model to explain the variation in the log maximum load of *Cryptosporidium* in raw water.

Variable	Coefficient	t[1]	*P*
Sheep excreta load	0.116	3.06	0.003
Sheep pens or cattle byres	0.082	2.98	0.004
Pig farms in catchment	0.784	2.46	0.016
High numbers of birds	0.178	2.06	0.044
No turbidity monitor on intake	0.19	2.83	0.006

[1] The test statistic **t** is the ratio of the Coefficient to the Standard Error of the respective predictor. The **t** value follows a standard normal distribution.

Variables derived from spatial datasets that remained in either of the final models were scored for entry into the revised raw water risk assessment based on their predictive power (by inspecting the z or t statistics and the associated *P* values) compared to other variables in the multivariate model. Catchment area was scored as 2 if ≤100ha and 8 if >100ha; greatest 5-day precipitation was scored as 2 if ≤90mm and 6 if >90mm, average slope was scored as 2 if ≤10% and 10 if >10% and sheep excreta load was scored as 2 if ≤4kg ha^{-1} of forage and 8 if >4kg ha^{-1} of forage. A catchment risk score was calculated for each WTW by summing the scores for the variables that remained in either of the final models. The variables in the revised RA and their final optimal scoring are shown in Appendix 2. Final risk bands that maximised the area under the ROC curve were: 0-28 (low risk), 29-35 (moderate risk) and >35 (high risk). The resulting ROC curve (Figure 18) had an area of 0.81 (95% C.I. 0.68-0.94), which indicates that the performance of the revised catchment risk assessment is good according to the academic points system; a large improvement on the performance of the existing RA. Logistic regression with a binary outcome demonstrated increased odds of raw water being positive at medium risk sites (OR=5.25, 95% C.I. 1.18-23.46, *P*=0.030) and high risk sites (OR=33, 95% C.I. 3.48-312.6, *P*=0.002) compared to low risk sites. The distribution of WTWs with raw water failures and non-detects between risk bands is shown in Table 14.

Table 14. The distribution of WTWs between catchment risk bands by whether or not their raw water failed the PCV during the study period.

Catchment Risk Score	Non-Detects				Positives				Total
	Count	% of non-detects	% of risk band	Mean max. oocyst load	Count	% of positives	% of risk band	Mean max. oocyst load	
0-28	7	58	50	0	7	12	50	1.33	14
29-35	4	33	16	0	21	34	84	1.30	25
>35	1	9	3	0	33	54	97	1.52	34
Total	12	100	16	0	61	100	84	1.42	73

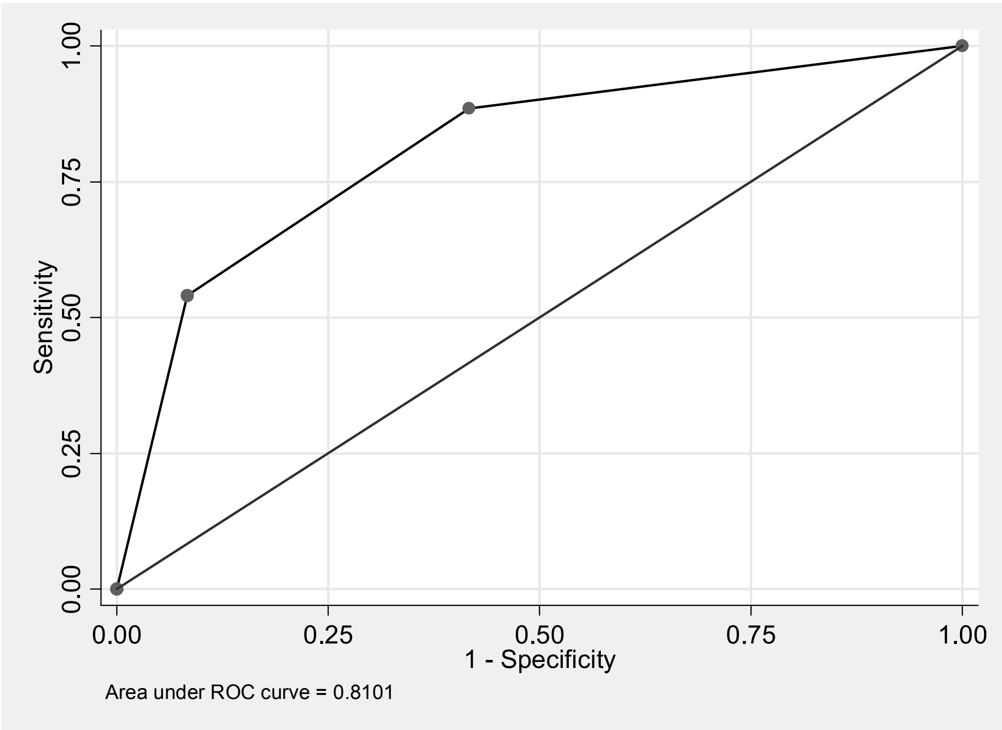


Figure 18. ROC curve showing the performance of the revised raw water risk band to predict *Cryptosporidium* occurrence in raw water.

When excluding non-detects, GLM logistic regression with the number of samples positive as the outcome variable demonstrated a significantly greater frequency of occurrence of positives at medium risk (coeff=0.65, z=2.14, P=0.032) and high risk (coeff=1.40, z=4.91, P<0.001) sites compared to low risk sites, and at high risk sites compared to medium risk sites (coeff=0.75, z=6.03, P<0.001). The mean log (max. oocyst load x 100) was greater for high risk catchments compared to medium and low risk when excluding non-detects (Figure 19), but this difference was not statistically significant.

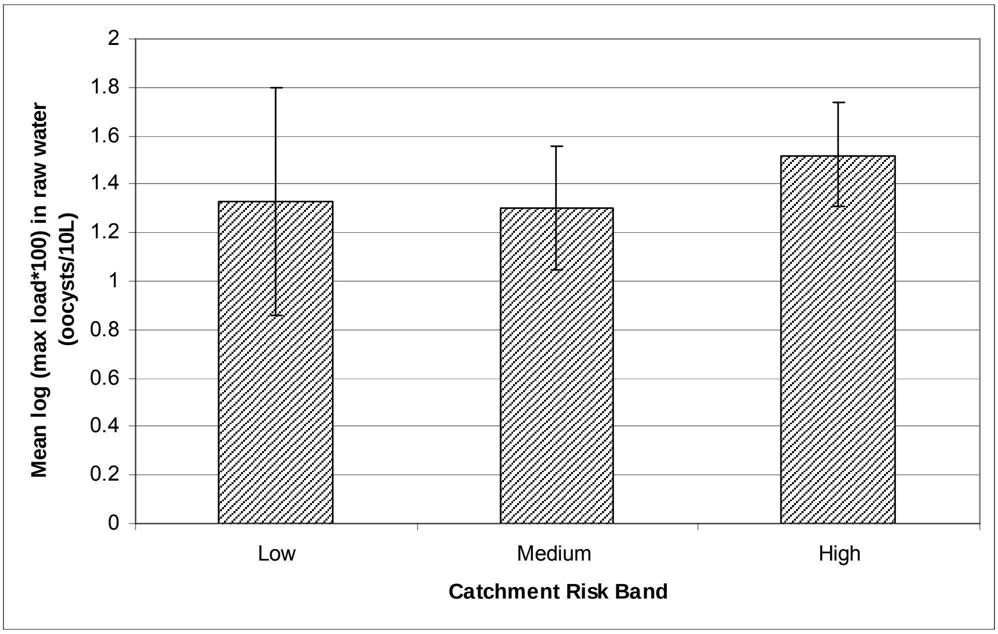


Figure 19. The mean of the log of the maximum oocyst load (x100) in positive raw water by catchment risk band. Error bars represent 95% confidence intervals.

A Spearman's correlation matrix of the variables in the revised raw water risk assessment is in Table 15. This shows that larger catchments are more likely to have a higher sheep excreta load and large numbers of birds; greatest 5-day precipitation has a positive association with slope, as does water source type; and water source types that are considered higher risk are more likely to have a turbidity monitor on intake.

Table 15. Spearman's correlation matrix of the variables in the revised raw water risk assessment. Correlation coefficients greater than 0.2 are shown in bold type.

	Log Area	Greatest 5-day precip.	Slope	Birds	Lambing / Calving	Water source type	Sheep excreta load	Sheep pens	Pig farms	Turbidity monitor
Log Area	1									
Greatest 5-day precip.	0.0091	1								
Slope	0.0491	**0.3448**	1							
Birds	**0.2023**	**-0.4051**	-0.0754	1						
Lambing/ Calving	0.0986	0.1067	0.1056	**0.2331**	1					
Water source type	-0.0564	0.1254	**0.3129**	-0.1260	0.0454	1				
Sheep excreta load	**0.3287**	**-0.2377**	-0.1714	**0.3739**	**0.2717**	**-0.3287**	1			
Sheep pens	**0.3795**	-0.1911	0.0202	**0.4414**	**0.2289**	0.0400	**0.2580**	1		
Pig farms	0.0985	-0.1063	-0.0502	0.1438	0.0374	0.1313	0.0964	0.1635	1	
Turbidity monitor	-0.1242	0.1329	**0.3108**	-0.1236	-0.0599	**0.5413**	-0.1730	-0.0336	-0.1267	1

4.5.2 Final Water Risk Assessment

4.5.2.1 Univariate analyses

The existing treatment risk assessment variables that were significant univariate predictors of *Cryptosporidium* frequency of occurrence or load are shown in Table 16. The catchment risk band derived in 4.4.1 was calculated for each of the 216 WTWs in the analysis sample, and entered as a covariate in each regression as a surrogate for the frequency of occurrence and load of *Cryptosporidium* in the raw water entering the WTW. Catchment risk was a significant predictor of final water prevalence or load in all models. The Water Treatment variable (section 8 of the RA) was a significant univariate predictor of *Cryptosporidium* frequency of occurrence (Coeff.=0.140, $P<0.001$) and load (b=0.032, $P<0.001$), and was also entered as a covariate in all subsequent regression models. Of the 216 WTWs in the analysis sample, 52 (24%) had membrane filtration on the SE list, 57 (26%) had coagulation followed by DAF/ sedimentation & filtration, 5 (2%) had slow sand filtration, 21 (10%) had coagulation followed by rapid gravity or pressure filtration, 3 (1%) had membrane filtration not on the SE list, cartridge/kalsep filtration or filtamat, 56 (26%) had simple sand filtration and the remaining 22 (10%) had either simple disinfection or microstraining.

Table 16. Existing treatment RA variables that were significant univariate predictors of either of the *Cryptosporidium* outcomes along with their regression coefficients and significance level.

Section of RA	RA Variable	Type of filtration applicable to	Frequency of Occurrence		Log Maximum Load	
			GLM Coeff.	*P*	OLS Coeff.	*P*
9.1-9.5	Turbidity meter on filter	RGF or Pressure	0.172	<0.001	0.032	0.034
9.6-9.8	Final water turbidity meter	RGF or Pressure	0.289	<0.001	0.058	0.016
9.10-9.12	Residual coagulant monitor	RGF or Pressure	0.108	<0.001	0.042	0.002
9.13-9.14	Routine sampling of residual coagulant	RGF or Pressure	0.563	<0.001	0.086	0.014
9.15-9.16	Turbidity of backwash supernatant monitored	RGF or Pressure	0.237	<0.001	0.078	0.034
9.17-9.21	Turbidity meter on filter	SSF	0.253	<0.001	0.053	0.048
9.22-9.24	Final water turbidity meter	SSF	0.540	<0.001	0.136	0.024
9.26-9.28	Matured	SSF	0.358	<0.001		
9.29-9.31	Monitored and alarmed	MF	0.105	<0.001		
9.32	Alarmed particle counter	MF	0.157	0.047		
10.6	Inspection and remedial work	RGF or Pressure	0.712	<0.001		
10.7	Air scour and backwash maintained	RGF or Pressure	0.785	<0.001	0.105	0.004
11.1-11.2	Control manuals	All	0.68	<0.001		
11.5-11.6	Slow start facility	RGF or Pressure	0.106	<0.001		
11.10-11.11	Backwash and sludge supernatant disposal	Most	0.115	0.001		
11.14-11.15	Design capacity	All	0.127	<0.001	0.089	<0.001

4.5.2.2 Multivariate analyses

The final minimal adequate models that best predicted the frequency of occurrence or load in the raw water are shown in Table 17 and Table 18. Whether or not the final water had failed the turbidity PCV in the study period was not a significant risk factor for either the frequency of occurrence or the load of *Cryptosporidium* in the final water, and neither was the maximum NTU.

Table 17. Variables that remained in the minimal adequate model to explain the variation in the frequency of occurrence of *Cryptosporidium* in final water.

Section of RA	Variable	Type of filtration applicable to	Coefficient	z	*P*
8.1-8.10	Treatment	All	0.127	17.81	<0.001
9.6-9.8	Final water turbidity meter	RGF or Pressure	0.335	9.21	<0.001
9.13-9.14	Routine sampling of residual coagulant	RGF or Pressure	0.476	6.29	<0.001
9.17-9.21	Turbidity meter on filter	SSF	0.078	3.6	<0.001
9.22-9.24	Final water turbidity meter	SSF	0.319	7.79	<0.001
9.26-9.28	Matured	SSF	0.158	5.16	<0.001
9.29-9.31	Monitored and alarmed	MF	0.081	2.67	0.007
10.6	Inspection and remedial work	RGF or Pressure	0.299	2.12	0.034
11.1-11.2	Control manuals	All	0.812	3.7	<0.001
11.14-11.15	Design capacity	All	0.065	2.8	0.005
	Catchment risk score	All	0.576	10.25	<0.001

Table 18. Variables that remained in the minimal adequate model to explain the variation in the log maximum load of *Cryptosporidium* in final water.

Section of RA	Variable	Type of filtration applicable to	Coefficient	t	*P*
8.1-8.10	Treatment	All	0.027	8.41	<0.001
9.10-9.12	Residual coagulant monitor	RGF or Pressure	0.039	3.06	0.002
9.22-9.24	Final water turbidity meter	SSF	0.134	2.36	0.019
11.14-11.15	Design capacity	All	0.084	3.53	0.001
	Catchment risk score	All	0.178	4.67	<0.001

A treatment risk score was calculated for each WTW by summing the scores for the variables that remained in either of the final models. A total score was calculated by summing the catchment risk score and the treatment risk score. The variables in the revised RA and their final optimal scoring are shown in Appendix 2. Scores were altered for certain variables in the RA based on expert opinion and their power as predictors in the final statistical models, however altering the scores did not improve the model fit, hence the existing RA scoring was used. Final risk bands that maximised the area under the ROC curve were: 0-16 (low risk), 17-28 (moderate risk) and >28 (high risk). The resulting ROC curve (Figure 20) had an area of 0.81 (95% C.I. 0.75-0.86), which indicates that the performance of the revised surface water risk assessment is good according to the academic points system; a small improvement on the performance of the existing RA. Logistic regression with a binary outcome demonstrated increased odds of final water being positive at medium risk sites (OR=3.93, 95% C.I. 1.89-8.20, $P<0.001$) and high risk sites (OR=27.53, 95% C.I. 11.33-66.93, $P<0.001$) compared to low risk sites. The distribution of WTWs with raw water failures and non-detects between risk bands is shown in Table 19.

Table 19. The distribution of WTWs between final risk bands by whether or not their final water failed the PCV during the study period.

Final Risk Score	Non-Detects				Positives				Total
	Count	% of non-detects	% of risk band	Mean max. oocyst load	Count	% of positives	% of risk band	Mean max. oocyst load	
0-16	61	60	79	0	16	14	21	0.39	77
17-28	32	31	49	0	33	29	51	0.43	65
>28	9	9	12	0	65	57	88	1.00	74
Total	102	100	47	0	114	100	53	0.75	216

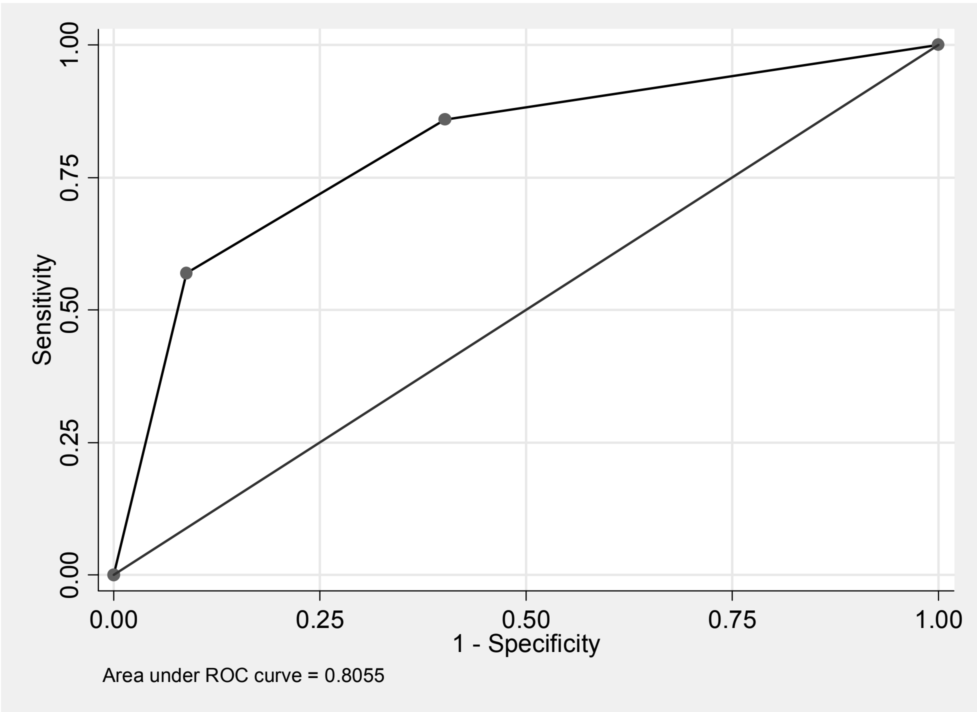


Figure 20. ROC curve showing the performance of the revised final risk band to predict *Cryptosporidium* occurrence in final water.

When excluding non-detects, GLM logistic regression with the number of samples positive as the outcome variable demonstrated a significantly greater proportion of positives at high risk (coeff=1.64, z=8.85, *P*<0.001) sites compared to low risk sites. Similarly, when excluding non-detects, the mean log (max. oocyst load x 100) increased with increasing final risk (Figure 21), and was significantly greater for high risk compared to low risk sites (b=0.618, t=3.97, *P*<0.001).

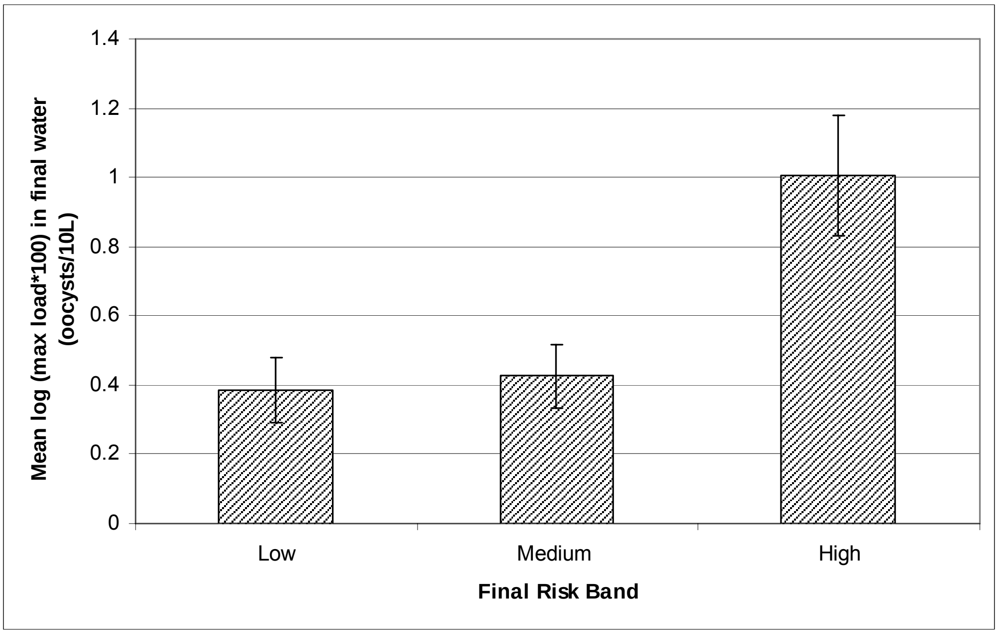


Figure 21. The mean of the log of the maximum oocyst load (x100) in positive final water by final risk band. Error bars represent 95% confidence intervals.

4.5.3 Groundwater Risk Assessment

Variables in the groundwater RA that, on initial inspection of the data, were potentially reasonable predictors of *Cryptosporidium* PCV failure in groundwater are shown in Figure 21 along with their GLM regression coefficients if significant.

Table 20. Variables in the groundwater RA that were potential predictors of *Cryptosporidium* in groundwater along with their regression coefficient and significance level.

Section Numbers	RA Variable	GLM Coeff.	*P*
1.3-1.4	Sheep/lamb density	0.106	0.005
1.5-1.6	Access to water source	0.182	0.001
1.7	Deer in catchment	NS	NS
2.1	Slurry spraying	NS	NS
2.5	Lambing or calving	NS	NS
4.1-4.6	Geology/ hydrology	NS	NS
5.1-5.4	Rapid by-pass of unsaturated zone	0.125	<0.001
6.1-6.5	Induced re-charge from surface water bodies	0.140	<0.001
7.1-7.3	Site drainage	0.147	<0.001
7.4-7.7	Location of headworks	0.039	0.024
8.1-8.3	Borehole construction/ integrity	NS	NS
9.6-9.7	Turbidity fluctuations	NS	NS
10.7-10.8	Control of flow increase	NS	NS

Variables that remained in the multivariate model were Rapid by-pass of unsaturated zone (coeff=0.105, z=4.84, $P<0.001$) and Site drainage (coeff=0.115, z=4.96, $P<0.001$). The final risk score obtained when summing these two variables was split into final risk bands: 0-9 (low risk), 10-19 (moderate risk) and >19 (high risk). The resulting ROC curve (Figure 22) had an area of 0.89 (95% C.I. 0.80-0.98). When summing the scores for all of the RA variables that were significant in univariate analyses (Table 20), the risk bands that maximised the area under the ROC curve were: 0-35 (low risk), 36-45 (moderate risk) and >45 (high risk). The resulting ROC curve had an area of 0.91 (95% C.I. 0.80-1.00), which indicates that the performance of the reduced ground water risk assessment is excellent according to the academic points system. This compares to a ROC curve area of 0.70 (95% C.I. 0.57-0.83) for the original risk assessment scoring. The distribution of WTWs with raw water failures and non-detects between the revised risk score categories is shown in Table 21. The variables in the revised RA and their final optimal scoring are shown in Appendix 2.

Table 21. The distribution of groundwater WTWs between final risk bands by whether or not their final water failed the PCV during the study period.

Final Risk Score	Non-Detects			Positives			Total
	Count	% of non-detects	% of risk band	Count	% of positives	% of risk band	
0-35	12	80	92	1	4	8	13
36-45	2	13	20	8	35	80	10
>45	1	7	7	14	61	93	15
Total	15	100	39	23	100	61	38

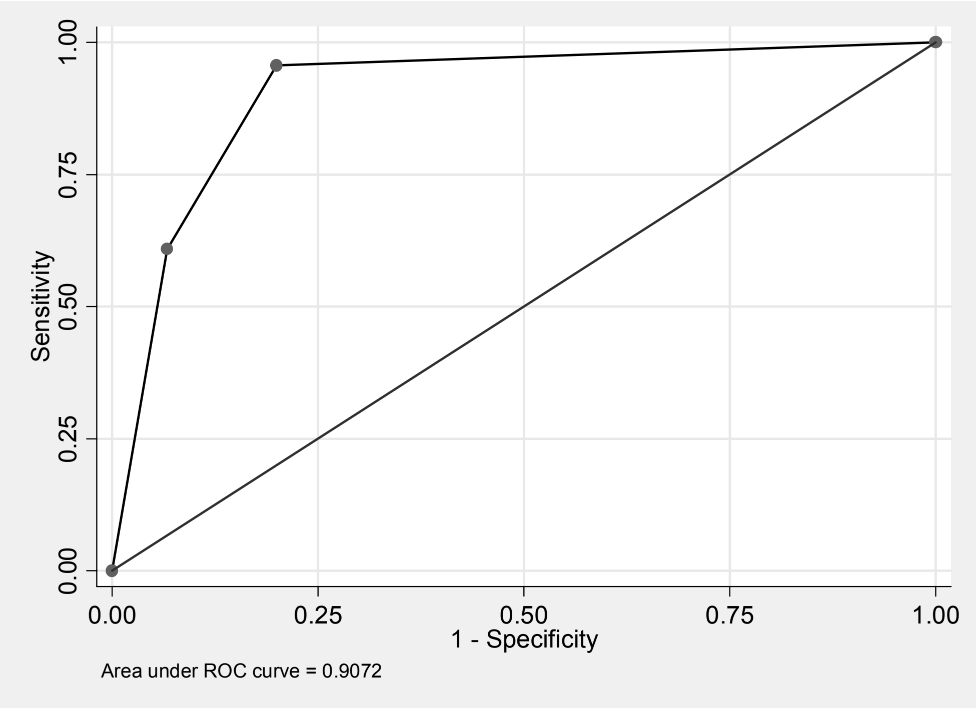


Figure 22. ROC curve showing the performance of the revised groundwater risk band to predict *Cryptosporidium* occurrence in ground water.

4.5.4 Spatial Variations in Risk

The spatial distribution of high, medium and low risk catchments are shown in Figure 23 and the numbers of high, medium and low risk catchments per region are detailed in Table 22.

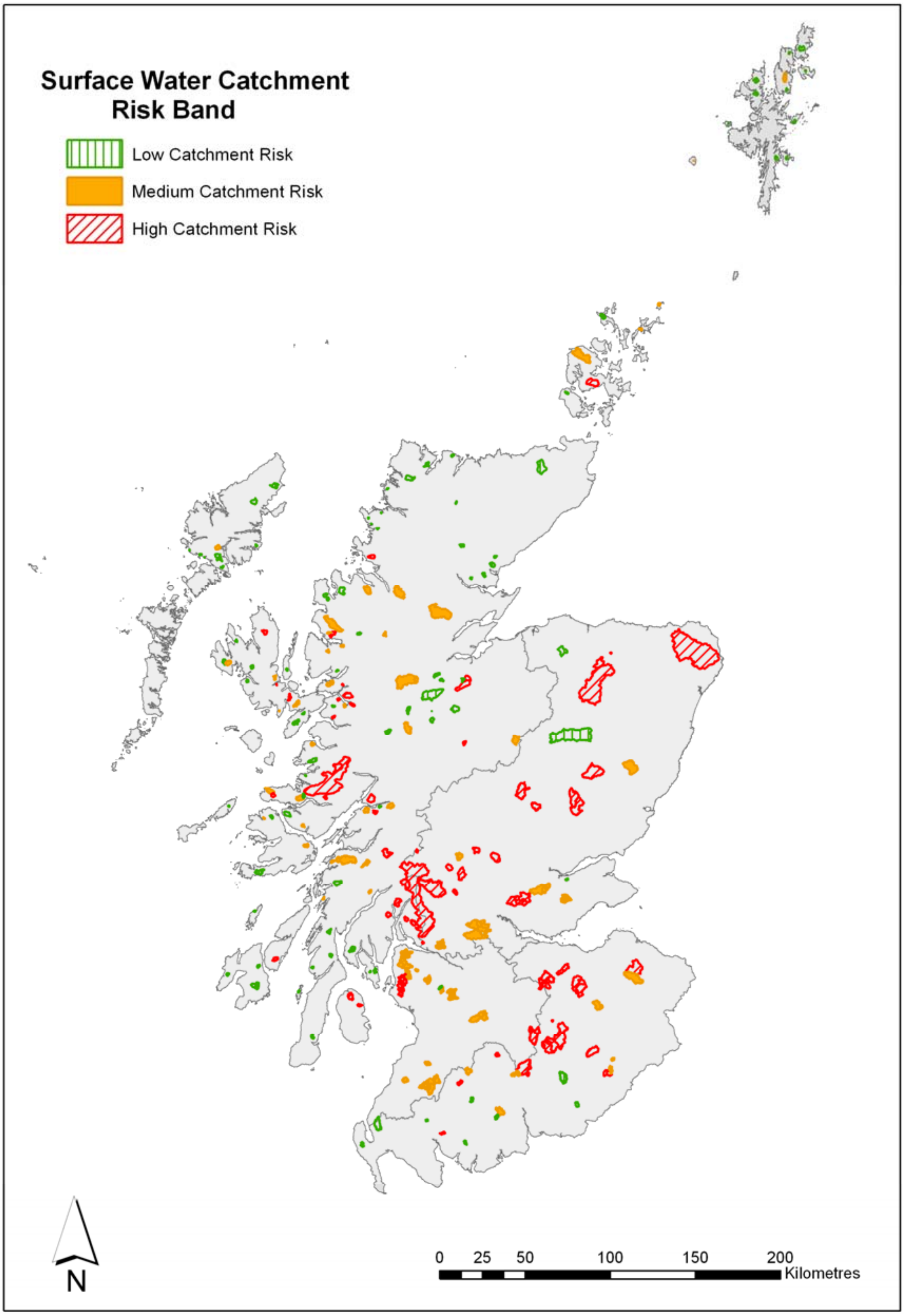


Figure 23. The spatial distribution of high, medium and low risk catchments that were used in the statistical analysis of risk assessment variables

Table 22. The distribution of catchments with low, medium and high risk assessment scores by operational region.

Region	Low Risk catchments	Med Risk catchments	High Risk catchments	Total Catchments
NW	85 (56%)	40 (26%)	28 (18%)	153
NE	3 (6%)	26 (50%)	23 (44%)	52
SW	1 (2%)	38 (65%)	20 (34%)	59
SE	9 (20%)	10 (23%)	25 (57%)	44

Median catchment risk varied significantly between regions (Kruskal Wallis χ^2 = 37.33, P<0.001). High risk catchments appeared to be concentrated in the NE and SE regions, which are the regions that had highest proportion of sites with positive final water (see Table 3).

The spatial distribution of high, medium and low risk final waters from WTWs in relation to the spatial distribution of catchment risk by region is shown in Figure 24, and the numbers of high, medium and low risk final waters per region are detailed in Table 23.

Table 23. The distribution of WTWs with low, medium and high final risk assessment scores by operational region.

Region	Low Risk final waters	Med Risk final waters	High Risk final waters	Total WTWs
NW	45 (33%)	38 (27%)	55 (40%)	138
NE	7 (23%)	18 (60%)	5 (17%)	30
SW	14 (64%)	7 (32%)	1 (4%)	22
SE	9 (35%)	6 (23%)	11 (42%)	26

Median final risk varied significantly between regions (Kruskal Wallis χ^2 = 9.88, P=0.011). WTWs with highest risk final waters were concentrated in the NW and the SE. WTWs in the SW had the highest proportion of low risk final waters. In the NW, the risk band into which the WTW was classified was generally higher for final waters than for raw waters, implying that the treatment process in the NW may not be sufficient. The NW also had the highest proportion of positives in final water samples (see Table 4. Numbers of raw and final surface water samples taken per operational region and the percentage of these that were positive for *Cryptosporidium*.). In the other operational regions, the treatment process appeared to lower or not alter the final risk band into which the WTWs were classified.

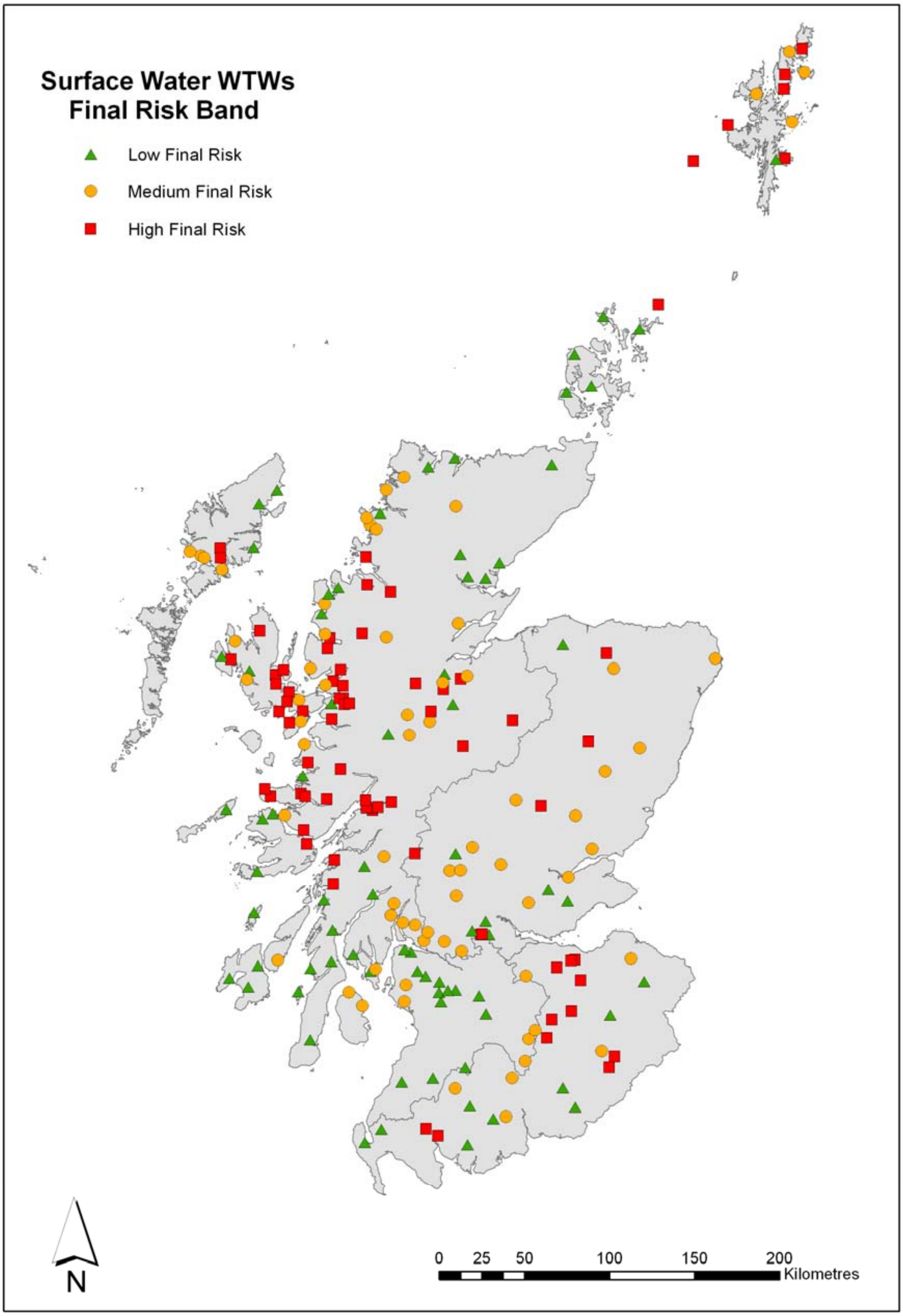


Figure 24. The spatial distribution of WTWs with high, medium and low risk final water.

The relationship between the catchment risk band and the final risk band into which the WTWs were classified across all regions is shown in Table 24. The majority (52%) of WTWs with low catchment risk also had a low final risk, however 19 (21%) of catchments with low catchment risk had high final risk, indicating that a level of treatment proportional to the catchment risk may not be sufficient in many cases. Of the WTWs with high catchment risk, 34 (54%) were at reduced final risk due to the performance of the treatment.

Table 24. The numbers of WTWs in each combination of catchment risk band and final risk band.

		Catchment Risk			
Final Risk		Low	Medium	High	
	Low	46	28	1	**75**
	Medium	24	12	33	**69**
	High	19	24	29	**72**
		89	**64**	**63**	**216**

4.6 Discussion

4.6.1 Raw Surface Water Risk Assessment

The existing catchment risk assessment for surface water does not perform well when used to predict the occurrence of *Cryptosporidium* in raw water, therefore an attempt was made to improve its predictive power. This was done by incorporating catchment-level data from available spatial datasets, excluding variables that explained little of the variation in the *Cryptosporidium* outcome, altering scores that were attributed to risk/protective factors and changing the cut-off scores for assigning WTWs to risk categories. This process resulted in a much improved catchment risk assessment that was a good predictor of *Cryptosporidium* occurrence in raw water. Of the non-detects, 58% were classified as low risk, and of the positives, 54% were classified as high risk (Table 14). The improved risk assessment was also a good predictor of the number of occurrences of PCV failure in positive raw waters when taking into account the sampling frequency.

The variables that remained in the final models, and were thus incorporated into the revised risk assessment, consisted of both physical characteristics of the catchment and the activities that occur in the catchment. Larger catchments appeared to be at higher risk, possibly due to there being a greater likelihood of a source of *Cryptosporidium* being present in the catchment. Catchments with a higher greatest 5-day precipitation total were also at higher risk. Surface run-off is more likely in areas where there are high volumes of rainfall in a short space of time, which in turn increases the likelihood of oocysts on the land being washed into surface waters. Interestingly, although the average annual rainfall had a positive association with the frequency of occurrence of *Cryptosporidium* in raw water in univariate analysis, it had a negative association with the maximum oocyst load. This suggests that a high amount of rainfall has a dilution effect, so that even though increased rainfall increases the likelihood of *Cryptosporidium* entering surface water, it decreases the concentration of oocysts in the raw water. It is likely that the most epidemiologically risky catchments are those that are drier on average, but have occasional high rainfall events, leading to the flushing out of a build-up of oocysts on the land. This result is also an indication that the abundance of oocysts in the catchment is source-limited, since if it were not, high loads would be expected in catchments with a high annual average rainfall. If the risk is source limited, reducing the abundance of the source (e.g. livestock) in the catchment could reduce the risk. In relation to the risk of oocysts entering surface water, catchments with a greater average slope had a higher frequency of occurrence of *Cryptosporidium* in raw water on average. The correlation matrix (Table 15) shows that steeper catchments are also more likely to have higher volumes of rainfall, although the association was not strong (r=0.34).

A number of variables relating to the presence of farmed sheep in the catchment were associated with either an increased frequency of occurrence or load of *Cryptosporidium* in the raw water; namely the sheep excreta load, the presence of sheep pens or cattle byres, and the occurrence of lambing or calving in the catchment. Neonatal ruminant livestock have been found to be the primary reservoirs of *C. parvum*, and there is also seasonal reporting of cryptosporidiosis in ruminants, with peaks occurring during lambing or calving (MAFF, 2000). The most prevalent species in the speciated sample (*C. andersoni*) is associated with bovines (Xiao *et al*., 2004), however none of the variables relating to cattle density or excreta load were significant predictors of *Cryptosporidium* outcome. A number of studies have derived statistical relationships between water quality and catchment characteristics relating to agricultural practices, including the percentage of the catchment under improved pasture (Kay *et al*., 1999) and the density of grazing livestock (Wilkinson *et al*., 1995). These models do not take into account the estimated volume of manure and the average concentration of oocysts in manures from the various species and ages of livestock. ADAS' Manure Management Database (MMDB) was used to calculate the quantity of excreta expected to be voided to land within the catchment by livestock type and age. Sheep and lamb excreta load were both significantly associated with maximum oocyst load in raw water, and it is probably no coincidence that sheep have the highest documented prevalence of *Cryptosporidium* oocysts in their manure than any other type of livestock (Hutchison *et al*., 2004).

The presence of pig farms and high numbers of birds in the catchment were both significant predictors in one or both of the final multivariate models, and also represent potential reservoirs of *Cryptosporidium*. Pigs infected with cryptosporidiosis have particularly high concentrations of oocysts in their faeces (58 oocysts/g on average in fresh faeces - Hutchison *et al*., 2004), therefore pig slurry spread to agricultural land or voided directly on outdoor pig farms is likely to be a higher risk in terms of oocyst concentration than other livestock manures. Birds are most commonly infected with the species *C. baileyi* (Xiao *et al*., 2004), which comprised just one of the 325 speciated samples, but zoonotic *C. parvum* has also been isolated from the faeces of wild Canada geese (Graczyk *et al*., 1998). *C. parvum* oocysts cannot cause infection in birds, but can pass through their gastrointestinal tract whilst maintaining their infectivity, thus wild birds, particularly water fowl, have the potential to act as mechanical carriers of *Cryptosporidium* and disseminate oocysts to surface water bodies. An analysis of storm runoff from roofs in farmyards identified significant concentrations of faecal streptococci and phosphorous originating from wash-off of bird droppings (Edwards & Kay, 2008), which is another potential method of transport of oocysts to water courses.

The water source type was a strongly significant variable in the final multivariate model to predict the frequency of occurrence of *Cryptosporidium* in raw water, whereas the lack of a turbidity monitor on the intake was a risk factor for a greater oocyst load. Direct abstraction from rivers or burns is scored most highly in the RA, with lowland sources given more weight. Secure natural springs and other shallow underground sources with vulnerable soil/ geology were scored the same as lowland reservoirs, whereas upland reservoirs and underground sources with non-vulnerable soil/ geology were given the lowest scores. This broadly reflects either the potential for filtration of oocysts through porous media or the potential for faecal matter to enter a surface water. For river and burn intakes, having an alarmed turbidity monitor that was connected to telemetry on the intake reduced the risk, whereas having no turbidity monitor increased the risk. It is a key recommendation by Binnie Black & Veatch in their 2002 investigation for the Scottish Government into the implementation of the Directions, that on-line turbidity instruments be provided, with appropriate alarms, on the raw water supply for sites where changes in quality can occur relatively quickly, for instance following heavy rainfall. This recommendation is supported by the results of the statistical analyses performed here.

Some of the variables in the revised raw water RA were weakly correlated (see Table 15), suggesting that certain variables are more likely to occur together in a particular type of catchment (e.g. upland). Additive scoring for variables that typify a catchment may artificially inflate the risk score, but will also reduce the errors associated with the measurement of individual variables.

4.6.2 Final Surface Water Risk Assessment

The existing treatment and supply risk assessment for surface water is a fair predictor of *Cryptosporidium* occurrence in final water, but it has the potential to be improved by review of the variables in the model and their scoring. No additional variables were considered, with the exception of turbidity monitoring results, as the existing RA was thought to be comprehensive. Removal of variables that explained little of the variation in the *Cryptosporidium* outcome, altering scores that were attributed to risk/protective factors and changing the cut-off scores for assigning WTWs to risk categories resulted in an improved RA that was a good predictor of *Cryptosporidium* occurrence in final water. Of the non-detects, 60% were classified as low risk, and of the positives, 57% were classified as high risk (Table 19). The improved RA was also a good predictor of the number of occurrences of PCV failure in positive final waters when taking into account the sampling frequency, and the maximum oocyst load in positive final water. These two variables may be more important epidemiologically, as disease occurrence in humans is related to the challenge that they are exposed to, in particular the oocyst load (Teunis *et al*., 1997). Even though 14% of the final water positive sites were classed as low risk, these misclassified sites were likely to have a lower frequency of positives and a lower oocyst load than positive sites in the other risk categories, and may therefore pose a lower risk to human health.

During the assessment of the existing RA, the US Environmental Protection Agency (USEPA)'s Long Term 2 Enhanced Surface Water Treatment Rule (LT2) was considered as a simple alternative. In the USEPA Rule, the treatment process is given a certain number of log-credits depending on the type of treatment. The majority of plants treating surface water (in the US) use conventional treatment, which is defined as coagulation, flocculation, sedimentation and filtration, and achieve a *Cryptosporidium* removal efficiency of approximately 3-log. Consequently, conventional treatment works receive 3-log credit. Studies indicate that works using clarification processes other than sedimentation that are located after coagulation and prior to filtration can achieve performance equivalent to conventional works. As a result, any treatment train that includes coagulation/flocculation, clarification, and granular media filtration is regarded as conventional treatment. The clarification step must be a solid/liquid separation process. Direct filtration plants use coagulation, flocculation and filtration processes, but lack a sedimentation basin or equivalent clarification process. Results from studies demonstrate that sedimentation basins can achieve 0.5-log or greater removal, therefore direct filtration plants are awarded a 2.5-log credit. Slow sand filtration involves passing raw water through a bed of sand at low velocity and without prior coagulation. Diatomaceous earth filtration is a process by which a filtration medium is initially deposited onto a support membrane and medium as added throughout the operation to prevent the filter from clogging. For both processes, studies indicate that a properly operated filter can achieve removal efficiencies similar to those of conventional treatment, and therefore receive a 3-log credit.

There is some danger in placing log credits against treatment regimes. Allocating a 3-log credit to a conventional treatment works assumes that the operation of the treatment regime will be optimal at all times. Providing this is the case, even with high challenges of *Cryptosporidium* in the raw water, the risk can be managed. However, if treatment is compromised in any way, there will always be the risk of oocysts in the final water.

The scoring system in the Directions against the type of water treatment appeared to be in line with the USEPA log credit system, with a score of -10 approximately equivalent to a 3-log credit. The alteration of the treatment score depending on the level of monitoring and the operation of the works is also the most appropriate way of taking the operational aspect of the risk into consideration. It is for these reasons that the format of the existing RA was used.

The type of treatment was a strongly significant explanatory factor in all models. The variables relating to Rapid Gravity or Pressure filtration that remained in at least one of the multivariate models were the presence/absence of a final water turbidity meter and if this was alarmed, the presence/absence of a residual coagulant monitor and if this was alarmed, whether or not there was routine sampling and analysis of the water treatment process for residual coagulant, and whether or not filters had been drained, inspected and necessary remedial work had been carried out. The variables relating to Slow Sand filtration that remained in one or both of the multivariate models were the presence/absence of turbidity meters on the individual filters and on the final water and if these were alarmed, and whether or not the filters had been matured and the filtrate analysed for turbidity, total coliforms and *Cryptosporidium* during maturation. The only variable relating to Membrane filters that remained in the final model were whether or not they were monitored and alarmed for integrity. Other variables that remained in one or more of the final models as significant explanatory factors were the presence/absence of control manuals specific to the plant and whether or not the plant had been run above its design capacity for >10% of the time in the last 12 months.

Turbidity needs to be constantly monitored to be reliable, and alarmed meters connected to telemetry are necessary for remedial action to be taken quickly enough to prevent oocyst breakthrough into final water. Individual monitors placed on each filter outlet would be expected to give more detailed information on the performance of each filter, but final water turbidity meters warn of spikes in turbidity that indicates reduced combined filter performance. Even though the presence of alarmed turbidity monitors was a protective factor, neither the frequency of occurrence of oocysts nor the maximum load were associated with increases in turbidity in final water when tested as a binary variable for failure of the PCV, or as a continuous variable for maximum NTU. However, it is generally widely accepted in the UK that the consistent production of low turbidity water is key to minimising exposure to *Cryptosporidium* (Bouchier, 1998). In England and Wales, 0.1 NTU is the cut-off for PCV failure, and if it exceeds 0.2 NTU, action should be taken. In Scotland, the cut-off for PCV failure is 1 NTU, which implies that the water is naturally more turbid than in England and Wales. This may mask the detection of increases in turbidity that indicate increased risk of pathogen contamination, but the reasons are unclear as to why turbidity monitoring data has no association with *Cryptosporidium* monitoring data whilst the presence of an alarmed turbidity meter is protective. The relationship between turbidity and oocyst load or presence in Scotland warrants further investigation.

4.6.3 Ground Water Risk Assessment

The groundwater RA could not be optimised in the same way as the surface water due to the low sample size; however the distribution of risk scores for individual variables between positive and negative sites was investigated to identify potential predictors, followed by statistical analyses of these variables in the conventional way, but allowing variables that had statistical significance in univariate but not multivariate analysis to contribute to the scoring. This process resulted in an improved Risk Assessment that was an excellent predictor of *Cryptosporidium* occurrence in groundwater. Of the non-detects, 80% were classified as low risk, and of the positives, 61% were classified as high risk (Table 21).

The main problem with attempting to improve the groundwater RA, in addition to the small number of groundwater sites, was the small size of the catchments. The accuracy of the spatial data is likely to be too low if applied to groundwater catchments and was therefore not attempted. Reliance was placed on the data generated by visits to the catchments over any remotely captured data. The strongest predictors of *Cryptosporidium* detection in groundwaters were the likelihood of surface water transmission to groundwater, and the adequacy of site drainage. The biggest risk of contamination of groundwater is expected to arise from ingress of surface water, and these results support this. The proportion of the yield that is derived from recharge from surface water and the location of the headworks were also significant univariate predictors that are associated with the likelihood of surface water ingress. The two variables related to activities in the catchment that were significant univariate predictors were the density of sheep/lambs and access of livestock to surface water. As discussed previously, sheep can have a high prevalence of cryptosporidiosis, and if they also have access to surface water, this increases the likelihood of oocysts being disseminated in surface water and potentially contaminating groundwater.

4.6.4 Regional Variations

The mapping of catchments and WTWs by operational region and symbolising according to their risk band was informative, as it gave an indication of the parts of the country where catchments were at highest risk from contamination and also which regions appeared to have most effective water treatment for removal of *Cryptosporidium*. The NW had the highest proportion of catchments that were classed as low risk, however it also had a large proportion of WTWs that were classed as high risk in terms of their final risk score. This suggests that the risk of contamination of raw water is the lowest in this region, possibly due to fewer potential sources of the organism, but on average the treatment is probably not sufficient to remove any oocysts that do get into raw water. The SE had the highest proportion of catchments that were classed as high risk, possibly due to there being more potential sources of *Cryptosporidium*, for example livestock, in the catchments. This region also had the highest proportion of WTWs with a high final risk score, implying that treatment did not score low enough to reduce the risk in final water from a high load in raw water. The NE and SW had mostly medium risk catchments. The NE also had mostly medium risk final waters, but the SW had mostly low risk final waters, indicating that the treatment performance in the SW was the most efficient at removing oocysts from raw water. The distribution of final risk bands regionally was similar to the percentage of sites that had positive final water from monitoring data. When investigating the ability of the treatment process to lower the final risk band into which WTWs were classified across all regions (Table 24), it was apparent that applying a level of water treatment in proportion to the catchment risk was not always sufficient, whereas where the catchment risk was high but treatment effective enough to remove the challenge, the risk band into which the WTW was classified could be lowered.

4.6.5 Assumptions and Recommendations

4.6.5.1 Overall Risk Assessment

The new risk assessment scoring system has been statistically tested and in all cases is a good predictor of *Cryptosporidium* occurrence, fraction of samples that test positive, and maximum oocyst load in both raw and final water for surface and ground water. It is, however, an assessment of risk based on easily measureable catchment and treatment works variables and cannot predict *Cryptosporidium* contamination that occurs as a result of one-off events that could not be foreseen. It is often these types of incidents that cause spikes in *Cryptosporidium*, therefore regular monitoring of the catchment for high-risk sources, and regular monitoring of the treatment operation are potentially as important as the risk assessment in determining sampling frequency.

- Recommendation – regular monitoring of catchment and of works operation

4.6.5.2 Existing Risk Assessment Variables

For existing catchment level variables that remained in the final models, it was assumed that data that vary seasonally, such as whether lambing or calving occurs in the catchment, were collected at the appropriate time of year, or that the person responsible for the risk assessment has good knowledge of the catchment. It is not known what constitutes ‘high numbers of birds’ in the risk assessment, but when scoring this variable in future RAs, account should be taken of colonies of breeding water birds and migrating birds in addition to farmed birds.

- Recommendation – seasonal variables assessed at appropriate time of year

For the risk assessment relating to the treatment works, it is important to ensure that data is collected by an impartial surveyor to reduce any bias. Another recommendation, if this is not already common practice, is for a data log to be kept of the plant’s performance – for example if there have been any signs of cracking on the filters over the course of the year. If the annual risk assessment only considers the state of the filters or media at that single time point, it may not accurately reflect the performance of the plant over the whole year, whereas if records were kept, these could be consulted to assist accurate scoring.

- Recommendations –
- Impartial surveys of treatment performance
- Data log of plant performance

4.6.5.3 Water Monitoring Data

As already discussed, one of the major limitations to statistical analyses of the predictive power of risk assessment variables is the biased sampling for *Cryptosporidium*. By design, and necessarily due to the high cost of diagnostic tests, both raw and final waters are monitored more frequently at higher risk sites. In a purely experimental design to identify risk and protective factors, sampling would have been consistent between sites, and both raw and final water would have been monitored at every site. Although this approach is not feasible, an alternative could be considered to improve the assessment of the performance of treatment works in removing *Cryptosporidium* oocysts from raw water. As discussed, it is impossible to match spikes in raw water to the loads in the same water after it has undergone the treatment process, and it is not always practical to challenge a WTW with *Cryptosporidium* in order to test its performance. However, the use of a surrogate to assess treatment performance on a regular basis is relatively easy. Aerobic spore-bearing bacilli are easy to isolate and enumerate. They are present in raw waters in high numbers and during conventional water treatment a three to four log removal should occur consistently, irrespective of the raw water quality. CREH have developed a simple medium for the purpose of monitoring aerobic spore-bearing bacilli in raw and treated waters (Francis *et al*., 2001). Such routine monitoring can readily provide information about treatment performance, and could assist in further optimising the RA for the treatment process in future years.

- Recommendation – use a surrogate for routine monitoring of treatment performance

4.6.5.4 Hydrological Catchments

For the purposes of deriving additional catchment level variables, a one to one relationship was created between raw water sources (abstraction points) and catchments where this did not already exist. Where an abstraction point was linked to more than one catchment, it was assumed that the hydrological catchment upstream of the abstraction point was the one from which surface water was routinely sourced. The accuracy of the catchment boundaries and the point locations of the abstraction points were unknown, but most were hydrologically correct when overlayed on a DTM and a water courses/ water bodies coverage.

4.6.5.5 Agricultural census data

Agricultural census data were available as parish level statistics. The main difficulty that arises when aggregating or disaggregating data from one polygon to another is that the distribution of the livestock within the parish is not known. An estimate of their distribution can be made based on land cover data, for example dairy cows are only likely to be grazed on managed grassland. Even though the distribution of livestock is weighted to a certain extent by this approach, the assumption is made that the numbers of a certain livestock type within a parish, and subsequently a catchment, have an even stocking density across the agricultural land cover. This assumption is more than likely incorrect, but is the best estimate given the available data. In future Risk Assessment scoring, availability of farm-level data with point locations would give a more accurate estimate of livestock numbers within a catchment, however knowledge of where the livestock were grazed would also be needed, particularly where farms were large or land distant to the main farm buildings was used. The numbers of farms of each robust type were not estimated as there would be even more inaccuracies in determining their distribution within a catchment. The variables relating to livestock densities and subsequently manure loadings per catchment give an indication of their likely magnitude, but should be considered comparative rather than absolute.

- Recommendation – investigate new methods for obtaining more accurate livestock density estimates

4.6.5.6 Other spatial data

Soil, climate and linear feature data were all aggregated to a grid cell level and thus all had a certain degree of spatial imprecision. For catchment attributes that can have a high level of spatial variability, such as soil type and the density of linear features, higher resolution or more accurately captured data sources would provide a more realistic representation of risk in the catchment. Rainfall will be less variable within catchments, but climate data at 5km grid cell resolution may be too coarse to characterise the smaller catchments. Simple climate monitoring or rainfall radar predictions from the Met Office may improve the assessment of catchment risk.

- Recommendation – investigate higher resolution data sources for soil and climate

4.6.5.7 Implementation of the revised risk assessment

Scoring of variables in the revised RA has been kept within the range of the original scoring system, however due to the reduced number of variables within the revised RA overall scores are lower than in the original. The scores assigned to variables did not affect their performance in the RA, but drastically altering weights may affect the risk ranking of WTWs and subsequently may cause some to be assigned a different risk category. Minor alterations of the scoring system are not likely to affect the final risk categorisation, particularly if cut-offs are reassessed. Cut-offs can be altered at the discretion of the Scottish Government in proportion to the availability of monitoring resources, however the cut-offs suggested here represent the optimum in terms of predictive power. Population weightings have not been considered, since these relate to epidemiological risk rather than the risk of oocysts entering final water, but these weightings could be adapted to adjust the revised scoring. A case could also be made for increased monitoring frequency at times of peak *Cryptosporidium* prevalence (see Figure 7).

- Recommendations –
- Can alter cut-offs in line with resource available for monitoring if necessary
- Increase monitoring frequency for larger populations and higher seasonal risk

4.6.6 Conclusions

The objectives of this project were;

(a) To develop statistically tested enhanced risk assessment schemes for evaluating risk to drinking water supplies from contamination in source waters.

(b) To ensure the approach retains a user friendly input.

(c) To ensure that the risk assessment scheme fits within the overall risk assessment and drinking water safety plan framework developed for use in Scotland.

Statistically tested enhanced risk assessment schemes have been developed that are better predictors of *Cryptosporidium* occurrence, proportion of samples positive and maximum oocyst load than the existing schemes, for raw surface water, final surface water and groundwater. Cut-off scores for allocation of WTWs to risk bands were based on maximising the area under the ROC curve, but these classifications could be changed if necessary, depending on the resources available for monitoring. All of the variables in the enhanced RAs were either already in the existing RAs or are easily measurable from spatial datasets, therefore remain user friendly to score. The additional catchment level variables are mostly physical characteristics that are unlikely to change from year to year, with the exception of the sheep excreta load, which can be easily estimated using the MMDB if the approximate number of sheep in the catchment is known. Drinking water safety plans were developed by the World Health Organisation as a means of protecting drinking water quality by identifying and managing the risk from pathogens, chemicals and diffuse pollution from source to tap. *Cryptosporidium* constitutes one of these risks, and thus a validated risk assessment scheme for this pathogen can be consolidated with risk assessments for other drinking water contaminants that are hazards to public health. Many of the risk assessment variables, particularly those at the catchment scale, will also be common to other pathogens, diffuse pollution and chemicals.

5 References

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Appendix 1.

Existing surface and ground water RA variables and the numbers of WTWs assigned each score.

	Surface Water RA		
Section 1 - Animals on the Catchment	***outcome***	***N***	***score***
Cattle/calf density per ha forage	<=1	229	6
	>1	38	12
Sheep/lamb density per ha forage	<=6	207	6
	>6	60	12
Animals have direct access to water source	Yes	258	4
	No	8	-1
Deer in catchment	Yes	212	2
	No	47	0
Pig farms in catchment	Yes	7	2
	No	261	0
High numbers of birds	Yes	44	2
	No	216	0
Any other farmed animals/birds in catchment	Yes	20	1
	No	248	0
Section 2 - Agricultural Practices			
Slurry spraying	Yes	24	6
	No	244	0
Dung spreading	Yes	31	3
	No	237	0
Dung or slurry storage	Yes	16	3
	No	252	0
Sheep pens or cattle byres	Yes	42	6
	No	226	0
Lambing or calving on catchement	Yes	202	8
	No	66	0
Section 3 - Discharges			
Septic tanks for population	<=100	263	4
	>100	4	6
sewage works for PE	<500	259	4
	501-5000	0	5
	501-20000	7	6
	20001-50000	1	7
	>50000	0	8
Storm water outlets	Yes	12	2
	No	256	0
Abbatior/livestock market	Yes	6	2
	No	262	0
Section 4 - Water Source Type			
Type of water source	secure natural springs - vulnerable soil	5	4
	secure natural springs - non-vulnerable soil	1	1
	other shallow underground - vulnerable soil	11	4
	other shallow underground - non-vulnerable soil	1	2
	upland reservoir	138	2
	lowland reservoir	14	4
	upland river/bum - direct abstraction	93	6
	lowland river/burn - direct abstraction	5	8
Section 5 - Raw Water Aqueducts*			
Raw water aqueduct	vulnerable from contamination from farmland	13	8
	not vulnerable or no aqueduct	255	0
Section 6 - Catchment Inspections			
Catchment inspections carried out monthly	Yes	5	-3
	No	263	6
Procedures in place to deal with irregularities in catchment	Yes	268	-3
	No	0	0
Section 7 - Raw water intake management*			
Turbidity monitor on intake	none	77	3
	alarmed and connected to telemetry	22	-2
Intakes	shut automatically under poor WQ	1	-4
	shut manually under poor WQ	22	-1
	not shut under poor WQ	75	3
Section 8 - Water Treatment			
	simple disinfection	32	10
	microstraining	4	10
	simple sand filtration	64	8
	coagulation followed by DAF/ sedimentation & filtration	61	-10
	coagulation followed by rapid gravity or pressure filtration	34	-7
	slow sand filtration	6	-9
	membrane filtration on SE list	61	-16
	membrane filtration not on SE list	1	-2
	cartridge/ kalsep filtration	2	-2
	filtamat	2	-2

Section 9 - Monitoring (Rapid Gravity or Pressure Filters)*			
Turbidity meter on filter	with an alarm on telemetry	53	-5
	without an alarm on telemetry	7	0
	shared over several filters with an alarm on telemetry	21	-2
	shared over several filters without an alarm on telemetry	7	2
	none	1	10
Final water turbidity meter	with an alarm on telemetry	78	-2
	without an alarm on telemetry	1	2
	none	8	5
Particle counter to continuously monitor performance		0	-5
Residual coagulent monitor	continuously monitoring combined filtrate/ outlet	68	-5
	continuously monitoring combined filtrate/ outlet - not alarmed	7	-1
	none	8	5
Routine sampling of residual coagulent	Yes	76	-2
	No	3	2
Turbidity of backwash supernatant monitored	Yes	39	-2
	No	12	2
Section 9 - Monitoring (Slow Sand Filters)*			
Turbidity meter on filter	with an alarm on telemetry	1	-5
	without an alarm on telemetry	2	0
	shared over several filters with an alarm on telemetry	0	-2
	shared over several filters without an alarm on telemetry	0	2
	none	3	10
Final water turbidity meter	with an alarm on telemetry	5	-2
	without an alarm on telemetry	0	2
	none	2	5
Particle counter to continuously monitor performance		0	-5
Slow sand filters	matured and filtrate analysed	2	-4
	matured but filtrate not analysed	4	5
	not matured	0	15
Section 9 - Monitoring (Membrane Filtration)*			
Membrane plant	monitored and alarmed for integrity	17	-3
	monitored for integrity but not alarmed	19	0
	not monitored for integrity	24	10
Alarmed particle counter to continuously monitor membrane performance		7	-5
Section 10 - Rapid Gravity & Pressure Filter Works Performance*			
Final water turbidity increases by	>50% excluding normal backwash period	12	4
	<50% excluding normal backwash period	73	0
Media depth	below design critical level	10	6
	above design level with audit trail maintained	29	-2
Signs of cracking of filters		11	4
All filters drained, inspected and remedial work carried out in last year		20	-2
Air scour and backwash maintained and operating efficiently		86	-2
Section 11 - Treatment Works Operation			
Treatment works process control manuals	available	264	-1
	not available	1	1
Auditable action plans for deviations in quality	available	266	-1
	not available	0	1
Slow start facility on RG or pressure filters*	operational	63	-4
	none or not operational	29	4
RG or pressure filters*	run to waste after backwash	11	-6
	run to head of works after backwash	6	-4
	not run to waste or head of works after backwash	55	4
Backwash and/or sludge supernatant*	has to be recycled	41	2
	alternative disposal route available	68	-2
Water flow through plant whilst operational	has increased by >10% in <30 mins in last year	60	2
	has not increased by >10% in <30 mins in last year	201	-2
Plant run above design capacity	>10% of time in last year	33	4
	<10% of time in last year	223	0
Only applicable to some WTWs			

Section 1 - Animals on the Catchment	Ground Water RA outcome	N	score
Cattle/calf density per ha forage	<=1	29	6
	>1	10	12
Sheep/lamb density per ha forage	<=6	22	6
	>6	17	12
Animals have direct access to water source	Yes	22	4
	No	12	-1
Deer in catchment	Yes	19	2
	No	20	0
Pig farms in catchment	Yes	2	2
	No	37	0
High numbers of birds	Yes	4	2
	No	35	0
Any other farmed animals/birds in catchment	Yes	4	1
	No	35	0
Section 2 - Agricultural Practices			
Slurry spraying	Yes	18	6
	No	21	0
Dung spreading	Yes	17	3
	No	22	0
Dung or slurry storage	Yes	15	3
	No	24	0
Sheep pens or cattle byres	Yes	15	6
	No	24	0
Lambing or calving on catchement	Yes	26	8
	No	13	0
Section 3 - Discharges			
Septic tanks for population	<=100	36	4
	>100	3	6
sewage works for PE	<500	37	4
	501-5000	1	5
	501-20000	0	6
	20001-50000	2	7
	>50000	0	8
Storm water outlets	Yes	4	2
	No	35	0
Abbatior/livestock market	Yes	0	2
	No	39	0
Section 4 - Geology/ Hydrology			
Type	Sand & gravel flow aquifer - free draining/ restricted mineralogy soil cover	19	12
	Sand & gravel flow aquifer - impeded drainage/ rich mineralogy soil cover	6	8
	Sandstone & conglomerates flow aquifer - free draining/ restricted mineralogy soil	6	8
	Sandstone & conglomerates flow aquifer - impeded drainage/ rich mineralogy soil	5	4
	Limestone	2	12
	Igneous and metamorphic	1	12
Section 5 - Rapid by-pass of unsaturated zone			
Transmission of surgace run-off to groundwater	Known rapid	5	20
	Possible	12	15
	Unlikely	22	5
	Proven that there is none	0	0
Section 6 - Induced re-charge from surface water bodies			
Proportion of yield from recharge from surface water	Significant	9	20
	Small	12	15
	No evidence	17	10
	Proven only from groundwater	1	-20
	Infiltration to spring pipework system	0	20
Section 7 - Site drainage			
Site drainage	Poor with run-off collecting and ponding	4	12
	Good, but contours bring run-off towards borehole	16	8
	Good, contours falling away from borehole	19	0
Headworks	In outside chamber and/or below ground level, liable to flooding	6	12
	In outside chamber but sealed and dry	12	9
	Inside building with cover flush to floor or imperfectly sealed	0	6
	Inside building with completely sealed raised cover	16	-4
Section 8 - Borehole construction/ integrity			
Borehole casing integrity	known or suspected poor	2	12
	suspected, but not proven good	25	4
	proven good	12	-8

Section 9 - Treatment works performance and monitoring			
Abstraction point turbidity meter	with alarm on telemetry	15	-5
	without alarm on telemetry	2	0
	one for several abstraction points with alarm on telemetry	3	-2
	one for several abstraction points without alarm on telemetry	1	2
	none	18	10
Turbidity fluctuations	detected in final water from continuous monitoring	11	4
	no evidence from continuous monitoring	11	0
	treatment works shut down automatically by increase in turbidity	5	-4
Section 10 - Treatment works operation			
Process control manuals	available	39	-1
	not available	0	1
Action plans for dealing with deviations in water quality	available	36	-1
	not available	3	1
Record of actions/ audit trail	available	34	-2
	not available	5	2
Flow increase	not controlled e.g. by variable speed drive or similar	28	5
	controlled	10	-2
Plant run above design capacity	>10% of time in operation over last year	6	4
	<10% of time in operation over last year	33	0

Appendix 2.

Revised surface water and ground water risk assessments.

A1(1) Catchment Score

Where there is more than one source supplying a WTW, each source should be assessed individually and the highest score used to calculate the combined catchment and treatment & supply score and final weighted score.

Score must be entered for (1.1 or 1.2) Score 1.3, 1.4, 1.5, 1.6, 1.7 as appropriate.

Section 1	Animals on the Catchment	Score
1.1	Sheep excreta load <= 4kg per hectare of forage	2
1.2	Sheep excreta load > 4kg per hectare of forage	8
1.3	Deer in catchment	2
1.4	Pig farms in catchment	2
1.5	High numbers of birds	2
1.6	Lambing or calving on the catchment	8
1.7	Sheep pens or cattle byres	6
	Section 1 Total Score	

Section 2	Catchment Characteristics	Score
2.1	Catchment area <= 100 hectares	2
2.2	Catchment area > 100 hectares	8
2.3	Greatest 5-day precipitation <= 90mm	2
2.4	Greatest 5-day precipitation > 90mm	6
2.5	Average slope <=10%	2
2.6	Average slope >10%	10
	Section 1 Total Score	

One score only must be allocated.

Section 3	Water Source Type	Score
3.1	Secure Natural Springs - vulnerable soil / geology	4
3.2	Secure Natural Springs - non-vulnerable soil / geology	1
3.3	Other shallow underground sources - vulnerable soil / geology	4
3.4	Other shallow underground sources - non-vulnerable soil / geology	2
3.5	Upland reservoir	2

3.6	Lowland reservoir	4
3.7	Upland river or burn - direct abstraction	6
3.8	Lowland river or burn - direct abstraction	8
	Section 2 Total Score	

*Only score if raw water is directly abstracted from river or burn source. (4.1 or 4.2)

Section 4	Raw Water Intake Management (RIVER/BURN INTAKE ONLY)	Score
4.1	No turbidity monitor on the intake	3
4.2	Turbidity monitor on the intake which is alarmed and connected to telemetry	-2
	Section 4 Total Score	

	A1(1) Total Score	

A1(2) Surface Water Treatment and Supply Score

Treatment scoring: If there is more than one treatment process on-site, the highest scoring process ONLY should be used in this procedure.

One score only must be allocated.

Section 5	Water Treatment	Score
5.1	Simple disinfection only	10
5.2	Microstraining	10
5.3	Simple sand filtration (not slow sand)	8
5.4	Coagulation followed by DAF / Sedimentation and filtration	-10
5.5	Coagulation followed by Rapid Gravity or Pressure filtration only	-7
5.6	Slow Sand filtration	-9
5.7	Membrane filtration (membrane on Scot. Exec. List of products capable of removing or retaining particles > 1 micron diameter	-16
5.8	Membrane filtration (membrane not on Scot. Exec. List)	-2
5.9	Cartridge / Kalsep filtration	-2
5.10	Filtamat or equivalent	-2
	Section 5 Total Score	

For RGF or Press Filters only. Score (6.1 or 6.2 or 6.3)+(6.4 or 6.5 or 6.6)+(6.7 or 6.8)+6.9

Section 6 a	Treatment Works Monitoring - Rapid Gravity and Pressure Filters	Score
6.1	Final water turbidity meter with an alarm on telemetry	-2
6.2	Final water turbidity meter without an alarm on telemetry	2
6.3	No final water turbidity meter	5
6.4	Alarmed residual coagulant monitor continuously monitoring combined filtrate or works outlet	-5
6.5	Residual coagulant monitor continuously monitoring combined filtrate or works outlet but not alarmed.	-1
6.6	No residual coagulant monitor	5
6.7	Routine sampling and analysis of the water treatment process carried out at the WTW for residual coagulant	-2
6.8	No routine sampling and analysis of the water treatment process carried out at the WTW for residual coagulant	2
6.9	All rapid gravity and pressure filters on treatment works have been drained, inspected and necessary remedial work carried out as needed in last year	-2

For SSF's only. Score (6.10 or 6.11 or 6.12 or 6.13 or 6.14)+(6.15 or 6.16 or 6.17)+(6.18 or 6.19 or 6.20).

Section 6 b	Treatment Works Monitoring - Slow Sand Filters	Score
6.10	Each filter has a turbidity meter with an alarm on telemetry	-5
6.11	Each filter has a turbidity meter but without an alarm on telemetry.	0
6.12	One turbidity meter is shared over several filters with an alarm on telemetry	-2
6.13	One turbidity meter is shared over several filters but without an alarm on telemetry	2
6.14	No turbidity meter monitoring filter performance	10
6.15	Final water turbidity meter with an alarm on telemetry	-2
6.16	Final water turbidity meter without alarm on telemetry	2
6.17	No final water turbidity meter	5
6.18	Slow Sand filters matured and filtrate analysed for turbidity, total coliforms and Cryptosporidium during maturation.	-4
6.19	Slow Sand filters matured but no analysis carried out on filtrate	5
6.20	Slow Sand Filters not matured	15

For Memb Filtration only. Score (6.21 or 6.22 or 6.23)

Section 6 c	**Treatment Works Monitoring - Membrane Filters**	**Score**
6.21	Membrane plant monitored and alarmed for integrity	-3
6.22	Membrane plant monitored for integrity but not alarmed	0
6.23	Membrane plant not monitored for integrity	10
	Section 6 Total Score	
Score (7.1 or 7.2)+(7.3 or 7.4)		
Section 7	**Treatment Works Operation**	**Score**
7.1	Treatment works process control manuals specific to the works available	-1
7.2	Treatment works process control manuals specific to the works not available	1
7.3	Plant run above design capacity >10% of time in last 12 months	4
7.4	Plant run above design capacity <10% of time in last 12 months	0
	Section 7 Total Score	
	A1(2) Total Score	
	A1(1) + A1(2) Total	
	A1(3) Population Weighting	
Any changes that may have impacted population weighting? Example- Other works off line, mained out, Increased population from new developments.		
	Population Supplied	
	A1(3) - Population weighting	
	Final Weighted Surface Water Risk Assessment Score	
	A1(2) + A1(2) * A1(3)	

A2(1) Groundwater Catchment Score

Where there is more than one source supplying a WTW, each source should be assessed individually and the highest score used to calculate the combined Catchment and Treatment & Supply score.

Score must be entered for (1.1 or 1.2). Score 1.3, 1.4 as appropriate. If the density of sheep/lambs is unknown, 1.2 should be chosen.

Consideration should be given to seasonal farming practices on catchment

Section 1	- Animals on the Catchment	Score
1.1	Sheep / lamb density <= 6 animals / hectare of forage	6
1.2	Sheep / lamb density > 6 animals / hectare of forage	12
1.3	Animals have direct access to water source (incl. Feeder streams)	4
1.4	Fencing prevents any access to water source (incl. Feeder streams)	-1
	Section 1 Total Score	

One score only must be allocated. Note: If 2.4 is scored, documented evidence must be available to demonstrate that there is no transmission of surface water.

Section 2	Rapid by-pass of unsaturated zone	Score
2.1	Known rapid transmission of surface run-off to groundwater	20
2.2	Possible direct transmission of surface run-off to groundwater	15
2.3	Direct transmission of surface run-off unlikely	5
2.4	Proven that there is no transmission of surface run-off	0
	Section 2 Total Score	

One score only must be allocated.

Section 3	Induced re-charge from surface water bodies	Score
3.1	Significant proportion of yield derived from recharge from surface water	20
3.2	Small proportion of yield could be derived from recharge from surface water	15
3.3	No evidence of yield being derived from induced recharge from surface water	10
3.4	Proven that groundwater recharge is derived only from groundwater	-20
3.5	Infiltration to spring pipework system	20

	Section 3 Total Score	

Score (4.1 or 4.2 or 4.3) + (4.4 or 4.5 or 4.6 or 4.7)

Section 4	**Site Drainage**	Score
4.1	Poor site drainage with run-off collecting and ponding	12
4.2	Good site drainage, but contours tend to bring run-off towards borehole	8
4.3	Good site drainage with contours falling away from borehole, or no possibility of run-off collecting	0
4.4	Headworks in outside chamber and / or below ground level, liable to flooding or leaking structure	12
4.5	Headworks in outside chamber but sealed and dry	9
4.6	Flow headworks inside building with cover flush to floor or imperfectly sealed	6
4.7	Headworks inside building with completely sealed raised cover	-4
	Section 4 Total Score	

Total Groundwater Catchment Score

	A2(1) Total Score	

A2(3) Population weighting

Any changes that may have impacted population weighting? Example- Other works off line, mained out, Increased population from new developments.

Population Weighting = Supply Population Multiplied by 0.4 Log 10.

	Population Served	
	Population Weighting	

FWS = Catchment Score Multiplied by Population Weighting

	Final Weighted Groundwater Risk Assessment Score	