INTRODUCTION.

The magnitude of microbial hazards is known to vary highly over time, depending on the contamination sources and runoff during rainfalls. As the infection risk for different pathogens relate to their sources, an understanding of the relative impact of faecal matter from humans and animals is of high importance. For example, as cattle are known to be carriers of the parasitic protozoa Cryptosporidium parvum, tracking the transport of faecal matter from ruminants is valuable. Quantifying the relative impact of various faecal sources gives a basis for water management, especially in relation to control measures. Various microbial source tracking methods have been presented during recent years, and their usefulness in different regions such as in the Nordic countries needs to be tested. In Lake Rådasjön at the Swedish west coast, positive detections of Cryptosporidium spp. in the raw water have indicated that parasite sources in the watershed may represent a human health risk related to drinking water. The aim of this study was therefore to assess the microbial impact from human and animal faecal sources around this lake, during baseline and rain event conditions, using microbial source tracking as a complement to the analysis of traditional indicators and parasites.

MATERIALS AND METHODS.

From Lake Rådasjön about 60 000 people in Mölndal are supplied with drinking water during normal operation, and potentially up to 500 000 people in Gothenburg during shorter time periods. A microbial sampling program was undertaken in the watershed, including 23 locations in the lake and in connecting streams. Grab samples (up to 25 per location) were taken over the year 2008 and biweekly from April to August. Sampling locations represented a faecal impact from point sources (municipal and on-site sewer systems, stormwater effluents) and from diffuse sources (pastures with horses and cattle). Two areas were in focus for the monitoring program: the area nearby the raw water intake (8 sampling locations) and an animal settling area located on a peninsula on the eastern side of the lake (5 sampling locations). In addition, flow-weighted automated samplings during rain events were undertaken at one site of each of these two areas, including flow and precipitation measurements. Sediment samples were taken at 5 locations in a stream entering the northern shore nearby the raw water intake, and analysed for comparison with concentrations in the water phase.

The grab samples and flow weighted samples from totally four events were analyzed for indicator bacteria (total coliforms, E. coli, intestinal enterococci, sulfite-reducing clostridia) and somatic coliphages using standard methods. Parasitic protozoa Giardia and Cryptosporidium spp. were analysed at the raw water intake (15 m depth) and in the flow-weighted rain event samples. For microbial source tracking, analyses for Bifidobacteria on Human Bifid Sorbitol agar (HBSA) were performed on samples from 10 of the 23 locations in the watershed. In the analysis, yellow colonies were counted, as hypothesised to represent fresh faecal impact from humans, while the number of total and yellow aerobes was assessed for comparison. Additionally, filtered water samples were stored in freezer as will be considered for analysis later on, using a locally evaluated qPCR method to determine the levels of human and ruminant specific genetic markers from Bacteroides.

RESULTS AND DISCUSSION.

Low or non-detectable levels of E. coli and enterococci were observed at the raw water intakes at 8 and 15 meter depth in the lake, but high levels were detected at several locations within a radius of 300 m from these. One of these was the stream entering the northern shore, as influenced by upstream on-site sewers, stormwater from a residential area and surface runoffs from the nearby pasture carrying horses during summer. Consistently high levels were detected in another nearby stream, used as a recipient for upstream on-site sewers, with maximum levels of E. coli and intestinal enterococci at 1.3x10^6 MPN/100 mL and 3.0x10^6 CFU/100 mL respectively. Similarly high levels were also detected on a peninsula on the east side of the lake, probably resulting from manure from horses and cattle. Results for somatic coliphages and sulfite-reducing clostridia confirmed the variation between the different sampling locations.

From the flow-weighted event samplings in the stream north of the lake, elevated levels (median 12000 MPN/100 mL for E. coli) were detected in June 2008, after an extended dry weather period of 1.5 month. Moderate levels were registered during a moderate rainfall in October (median 225 MPN/100 mL), with high bacteria levels at the end of the event sampling period. In the event samplings at the pasture located east of the lake, surface runoff water was collected in perforated plastic pipes positioned at the ground. The event samplings at this site were performed in November, about one month after the cattle left the area. The highest levels of E. coli (350 MPN/100 mL) and clostridia (910 CFU/100 mL) were detected in the first flush of the first rain event (6 mm), while low levels of E. coli (median 19 MPN/100 mL) were registered during a higher rainfall (16 mm) in the same week.

Results from the analysis of Bifidobacteria were compared to the levels of traditional microbial indicators. The initial morphological classification in the analysis, based on the counts of yellow colonies anaerobically grown on the HBSA medium, overestimated the actual numbers of sorbitol-fermenting colonies, as only a minority of isolate yellow colonies were found to be strict anaerobes. Yellow obligate anaerobes, assumed to originate from humans and therefore indicating a likely influence of human faecal matter, were detected at 8 of 10 locations in the watershed. In contrast, no yellow obligate anaerobes were detected at locations characterized as only animal settlings. Results from analysis of specific genetic markers of Bacteroides, with lower detection limits expected, may give additional information as to what extent human and ruminant faecal matter may penetrate the raw water intake at 15 m depth.