PREVALENCE AND CONCENTRATION OF *CAMPYLOBACTER*, *E. COLI* O157 AND *CRYPTOSPORIDIUM* IN FECES OF WILD CATTLE, SHEEP AND DEER RANGING IN A WATER CATCHMENT AREA

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Aims

The Amsterdam water supply dune area is a water catchment area where rainwater and pretreated river water is stored in basins and then passed through dune soil to remove microorganisms and chemical contaminants. Subsequently, a cascade of treatment steps is used to enhance the quality and safety of the water. The catchment area is a nature area where grazing animals (sheep, cattle and deer) are introduced as a nature management tool. The presence of wildlife or livestock in the catchment area is a potential danger for spreading a wide range of zoonotic pathogens in the area and can potentially result in high loads of different pathogens in source water. Knowledge about the presence of pathogens in wildlife and livestock can help the water company to make decisions on how to design the water treatment plant and on how to manage the catchment area in such a way that contamination of source water by animals can be minimized.

Methods

Fecal samples were obtained from animals which were grazing in the Amsterdam water supply dunes. Fresh fecal samples (1-10 g) were collected at random locations from the ground in the dune area and stored at -20ºC until DNA was extracted. DNA was extracted using the “FastDNA Spin Kit for Soil” The elution step was repeated once to maximize yield and the volumes of both elution steps were pooled resulting in a suspension of 200 µl DNA.
All PCR analysis were performed in duplicate on 10 µl undiluted and (in duplicate) on 10 µl of 10-times diluted (in sterile PCR grade H_2O) extracted DNA..
The quantitative analysis of *E. coli* O157 was performed using the previously described O157BF2 and O157BR2 primers targeting the rfbE gene, a newly designed double labeled probe (O157Ti1) was used to detect the synthesis of *E. coli* O157 specific PCR fragments during the PCR reaction.
Primers and a double labeled probe, specific for the 16S rRNA gene of the *Campylobacter* genus were used for quantitative detection of *Campylobacter*.
An Inhibition and yield Control-plasmid (IC) was added to every sample.
E. coli O157 and IC specific reactions were performed as duplex PCR reactions detecting both PCR-targets simultaneously. Quantification was performed with the use of plasmid-DNA standards.
A previously described nested PCR procedure for the 18S rRNA was used to detect and genotype *Cryptospodidium*. Sequence analysis of the 18S rRNA fragment was performed on PCR-positive samples was performed for genotyping.

Results and conclusions

The recovery efficiency of IC varied from 35.6% (on average) in samples from cattle feces to 24.4% in samples from sheep feces and 15.5% in deer feces.

The presence and concentration of *E. coli* O157 and *Campylobacter* was determined in fecal samples from sheep (n=79), cattle (n=61) and deer (n=118) using qPCR. Only the presence (not the concentration) of *Cryptosporidium* in these samples was determined by nested PCR targeting the 18S rRNA gene of this organism. The results are summarized in table 1.
The highest *Campylobacter* prevalence was found in fecal samples from cattle (80.3%), the prevalence in sheep samples was considerably lower (19.0%) and lowest in fecal samples from deer (8.5%). The *Campylobacter* concentrations in fecal samples were high in samples from sheep (average 2.6 X 10^8 genome copies . g^{-1}), lower in cattle (average 8.9 X 10^7 genome copies . g^{-1}) and lowest in samples from deer (average: 3.4 X 10^6 genome copies . g^{-1}).
Compared to *Campylobacter*, the prevalence of *E. coli* O157 was much lower, *E. coli* O157 was detected in 14.8% of the fecal samples from cattle and 3.4% of fecal samples from deer. *E. coli* O157 was not detected in fecal samples from sheep. The concentration *E. coli* O157 in fecal samples from cattle (average 1.4 X 10^6 genome copies . g^{-1}) was higher then the concentration observed in fecal samples from deer (average 7.7 X 10^4 genome copies . g^{-1}).
Cryptosporidium was detected in 3 samples from sheep (3.8%), one cattle sample (1.6%) and eight fecal samples from deer (6.8%).
The sequences of the detected *Cryptosporidium* 18S rRNA PCR-fragments were determined and compared with published sequences to obtain information about the genotypes which are present in the fecal samples. Figure 1 shows a dendrogram based on a ClustalW multiple sequence alignment.
Sequences identical to previously described 18S rRNA sequences of the cervine genotype of *Cryptosporidium* were detected in three fecal samples from sheep. In the single *Cryptosporidium* positive sample from cattle, a sequence identical to a previously described 18S rRNA sequence of *Cryptosporidium parvum* was detected. Five different 18S rRNA sequences corresponding to different *Cryptosporidium* genotypes were detected in fecal samples from deer. One sequence (detected in three samples) was identical to the 18S rRNA sequence which was also detected in cattle and corresponds to *Cryptosporidium parvum*. A new 18S rRNA sequence, closely related to *Cryptosporidium parvum* (99% sequence similarity) was detected in one fecal sample from deer. A sequence identical to a published 18S rRNA sequence from *Cryptospordium muris* was detected in one sample, and a sequence identical to a published 18S rRNA sequence *Cryptosporidium bovis* was detected in one sample. Sequences identical to the 18S rRNA sequence of the deer-like *Cryptosporidium* genotype was detected in two samples collected on the same day were detected in four other fecal samples from deer.

The use of PCR methods makes it possible to screen for the presence of multiple pathogens after performing one universal DNA-extraction procedure making this an efficient approach. The use of qPCR for quantification of pathogens in fecal samples is a very practical and reliable alternative to culture methods also. Extraction methods that are capable to extract DNA from fecal samples with higher yield and less PCR-inhibition would be beneficial for both qualitative (lower detection limit) and quantitative (more accurate) measurements.

Table 1. The results of (q)PCR analysis of fecal samples from Sheep, Cattle and Deer for the presence of *Cryptosporidium* and the presence and concentration of *Campylobacter* and *E. coli* O157. *Campylobacter* and *E. coli* O157 concentrations are shown a the number of genome copies per gram feces (genome copies . g^{-1}).

Pathogen	**Sheep (n=79)**				**Cattle (n=61)**			
	Positive n (%)	**Average concentration in positive samples (genome copies . g^{-1})**	**Range (genome copies . g^{-1})**	**Median (genome copies . g^{-1})**	**Positive n (%)**	**Average concentration in positive samples (genome copies . g^{-1})**	**Range (genome copies . g^{-1})**	**Median (genome copies . g^{-1})**
Campylobacter	15 (19.0)	2.6 X 10^8	1.2 X 10^3 - 8.2 X 10^9	1.5X 10^7	49 (80.3)	8.9 X 10^7	9.2 X 10^1 -4.1 X 10^8	2.2 X 10^7
E. coli O157	0 (0.0)	<80	--	--	9 (14.8)	1.4 X 10^6	3.7 X 10^3 -1.2 X 10^7	5.5 X 10^4
Cryptosporidium	3 (3.8)	ND	ND	ND	1 (1.6)	ND	ND	ND
	Deer (n=118)							
Campylobacter	10 (8.5)	3.4 X 10^6	2.7 X 10^3 – 1.6 X 10^7	2.6 X 10^3				
E. coli O157	4 (3.4)	7.7 X 10^4	8.2 X 10^2 – 1.1 X 10^5	3.7 X 10^4				
Cryptosporidium	8 (6.8)	ND	ND	ND				

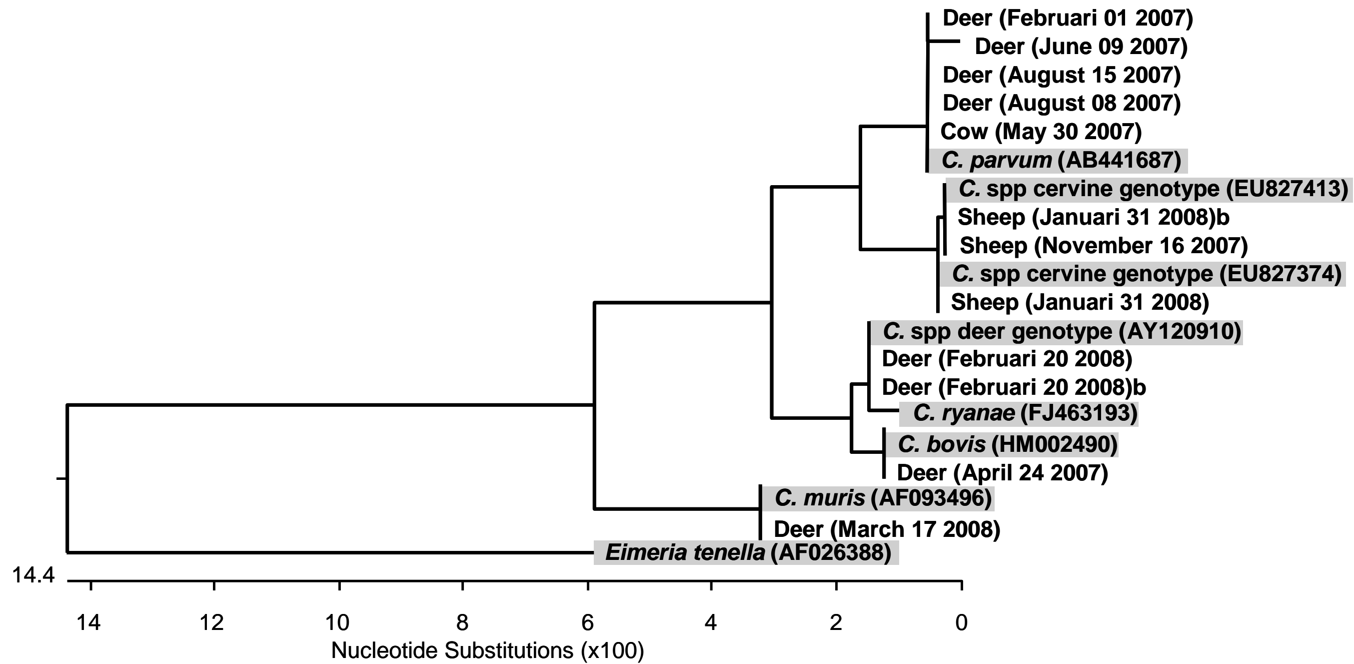

Fig. 1 Genetic relationship among published reference sequences and sequences detected in fecal samples with PCR, based on a ClustalW sequence alignment of partial 18S rRNA sequences. Reference sequences are boxed in grey and are shown with there accession numbers.