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short communication

DNA-based faecal source tracking of contaminated drinking water causing a large *Campylobacter* outbreak in Norway 2019

Lisa Paruch, Adam M. Paruch*, Roald Sørheim

Norwegian Institute of Bioeconomy Research (NIBIO), Division of Environment and Natural Resources, Fredrik A. Dahls Vei 20, 1433, Aas, Norway

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ABSTRACT

During June 2019, an outbreak of campylobacteriosis occurred in Askøy, an island northwest of Bergen, Norway. According to the publicly available records, over 2000 residents fell ill and 76 were hospitalised, and two deaths were suspected to be associated with *Campylobacter* infection. By investigating the epidemic pattern and scope, an old caved drinking water holding pool was identified that had been faecally contaminated as indicated by the presence of *Escherichia coli* (*E. coli*). Furthermore, *Campylobacter* bacteria were found at several points in the water distribution system. In the escalated water health crisis, tracking down the infectious source became pivotal for the local municipality in order to take prompt and appropriate action to control the epidemic. A major task was to identify the primary faecal pollution source, which could further assist in tracking down the epidemic origin. Water from the affected pool was analysed using quantitative microbial source tracking (QMST) applying host-specific *Bacteroidales* 16S rRNA genetic markers. In addition, *Campylobacter jejuni*, *Enterococcus faecalis*, *Clostridium perfringens* and Shiga toxin-producing *E. coli* were detected. The QMST outcomes revealed that non-human (zoogenic) sources accounted predominantly for faecal pollution. More precisely, 69% of the faecal water contamination originated from horses.

1. Introduction

Campylobacter is one of four global causative agents of diarrhoeal diseases (WHO, 2018); it has been considered as the most common bacterial pathogen to cause human gastroenteritis, as reported in the USA during 2004–2012, it has caused 1.3 million illnesses annually, 13,240 admissions to hospital and 119 deaths (Geissler et al., 2017). Among all species of the genus *Campylobacter*, two particular, *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), are the leading causes responsible for human infectious disease termed campylobacteriosis. The identified routes of transmission consist of the consumption of infected meat (e.g. undercooked poultry), contact with companion animals and livestock, drinking contaminated water and unpasteurised milk products. Campylobacteriosis outbreaks have been frequently reported worldwide: e.g. in the UK in 2013 and 2015 (annual incidence of 50–100 cases per 100,000 population), the USA in 2004–2012 (303,520 cases registered), Canada in 1990–2006 (annual incidence of 35.2 cases per 100,000 population), New Zealand in 2008 and 2016 (161.5 and 235.1 cases per 100,000 population, respectively), China in 2005–2009 (5–15 cases per 100 gastroenteritis) and Denmark in 2009–2010 (35 cases per 100,000 population) (Kaakoush et al., 2015). In Norway, according to the systematic case registration in the Norwegian Surveillance System for Communicable Diseases (MSIS, 2019), *Campylobacter* is the most common bacterial pathogen which causes human gastroenteritis. From 2000 to over 3000 infection cases were registered in the MSIS each year in the last two decades. Moreover, *Campylobacter* was identified as the most common bacterial pathogen that has resulted in all reported waterborne outbreaks in Norway between 1998 and 2002 (Jakopanec et al., 2008). However, in half of the outbreaks, the infective agent remained unknown. Such a challenge of pinpointing the source of infections could be somehow related to the notified weak host-association due to the rapid transmission of *C. jejuni* and *C. coli* between different hosts (Dearlove et al., 2016).

Campylobacter is a commensal member of the gut microbiota of many domesticated and wild animals. Thus, waterborne campylobacteriosis outbreaks are in close association with faecal water contamination (Pedati et al., 2019; Chukwu et al., 2019). Therefore, the origin of faecal pollution represents the most suspicious source of infection. As a result, tracking down the source of faecal contamination is of great importance, it should be the “first line” action to narrow the path of investigation and accelerate the downstream infectious source typing. Microbial source tracking techniques using real-time quantitative polymerase chain reaction (qPCR) based detection on host-

* Corresponding author.
E-mail address: adam.paruch@nibio.no (A.M. Paruch).

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associated genetic markers have been broadly adopted to determine the origins of faecal pollution (Zheng and Shen, 2018; Vadde et al., 2019). Most well developed and validated genetic markers are derived from *Bacteroidales* 16S rRNA genes, which exhibit high species distinction between variant hosts (Haramoto and Osada, 2018; Xue and Feng, 2019). Such a host-specific feature enables discriminating primary faecal sources of anthropogenic or zoogenic origins. Moreover, it could further identify specific hosts such as humans, ruminants, horses, swine, birds and others (Paruch et al., 2017; Mayer et al., 2018; Sowah et al., 2017; Staley et al., 2016).

In this study, we report the results of faecal source tracking conducted during the drinking water health crisis targeted in the Askøy municipality in June 2019, which happened to be by far the largest waterborne *Campylobacter* outbreak in history registered in Norway. The results are reported solely in the original version by the authors who were commissioned by the Askøy municipality to investigate the contaminated water by applying DNA-based markers for quantitative microbial source tracking (QMST) of faecal origin and pollution sources.

2. Incident description

An acute situation occurred on the 6th of June 2019, when dozens of residents of Askøy (a municipality with over 29,000 citizens, located on an island approx. 1 km northwest of Bergen in Hordaland County, Western Norway, Fig. 1) suffered from diarrhoea, abdominal pain and fever. Within the next few days, hundreds of people became sick with the same symptoms typical for food- or waterborne infections. Shortly afterwards, it was discovered that drinking water supplied to half of the island population was contaminated with faecal indicator bacteria *Escherichia coli* (*E. coli*). At the same time, more people were hospitalised, and Haukeland University Hospital in Bergen reported that *Campylobacter* bacteria were isolated from the stool samples of the patients. This released a clear alarm that the Askøy community was heavily hit by a large and severe waterborne outbreak of campylobacteriosis resulting in over 2000 sick people, among which 76 had to be hospitalised, and two deaths occurred which were highly suspected to be in close association with *Campylobacter* infection.

By investigating the pattern and scale of the epidemic, it was quickly located and confirmed that one aged mountain tunnelled drinking water holding pool (Høydebasseng HB 168 connected to the Kleppe Waterworks in Askøy distributing water to about 15,000 people, Fig. 1) caused the entire water health crisis. Analyses on the number of water samples collected from that pool revealed the presence of faecal indicator bacteria; thus chlorination was performed and a “boil water advisory” was implemented for six weeks on the island. On the 24th of June 2019, after emptying water from the holding pool, the entire cave was inspected, and laser scanned. These investigations discovered a number of cracks in the rocks of the cave through which the faecally contaminated water could have seeped into the pool. Notably, heavy rain episodes occurred right before the outbreak; this part of the country (with Bergen one of the rainiest city in Europe) is one the most precipitous area in Norway. This drinking water health crisis was widely reported in local and national media. It also gained worldwide attention and was described in both professional magazines and global news, e.g. in Sweden (VeterinärMagazinet, 2019), the Netherlands (Academic Medical Center, 2019), the UK (Sky News, 2019), the USA (Forbes, 2019), and China (Xinhua News, 2019).

3. Determining the origin and sources of faecal water contamination

Under the critical time pressure to tackle the water health crisis, several investigating directions (water system inspections, geographical/field observations, sampling rounds and laboratory studies) were set out to seek the source of the epidemic. The principal effort was dedicated to the identification of the primary source of faecal water contamination, which could further assist in tracking down the cause of the epidemic. The first step focused on determining if the contamination was of either an anthropogenic- or zoogenic-dominant origin. By this, the identification process was greatly simplified as one of the huge contamination sites could be excluded. Secondly, defining the particular origin of the faecal contamination allowed to narrow the focus on tracking the real pollution sources.

Determination of the faecal origin and pollution sources in the problematic water was conducted by the Norwegian Institute of Bioeconomy Research (NIBIO) utilising the developed methodological toolbox with DNA-based markers for QMST tests. NIBIO's toolbox comprises a microbial and molecular three-step testing procedure, as follows: 1) screening faecally polluted samples based on examination of *E. coli* concentration expressed as the most probable number (MPN)/100 ml, 2) tracking pollution sources in the screened samples (only these positive for *E. coli*) based on the detection and quantitation of host-specific *Bacteroidales* 16S rRNA genetic markers through qPCR, and 3) profiling faecal origin and sources based on the contribution percentage of the markers defined in the faecally polluted sample. The scientific background and processing techniques of this toolbox have been described in greater detail elsewhere (Paruch et al., 2019).

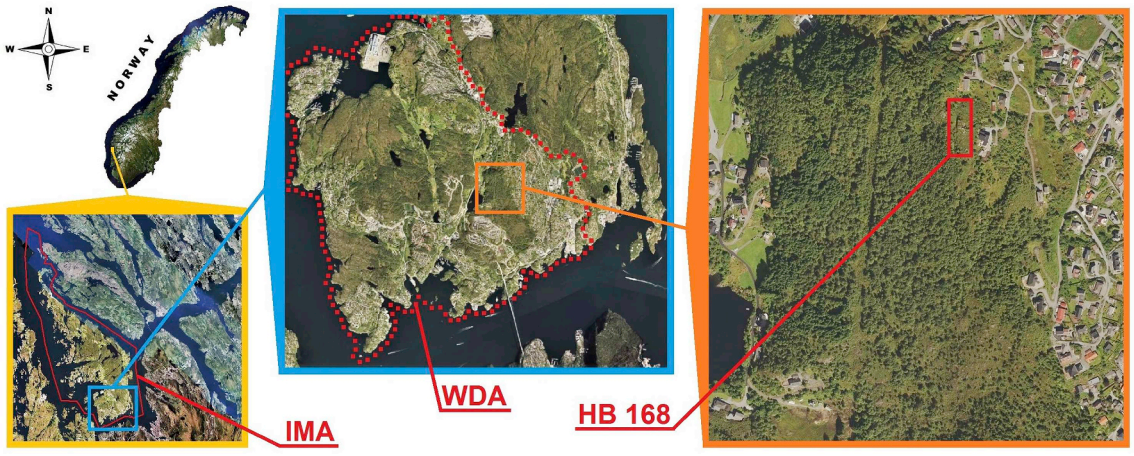

Fig. 1. Location of the island municipality of Askøy (IMA), the water distribution area (WDA) and the mountain tunnelled drinking water holding pool (HB 168). The satellite images were obtained from NIBIO's primary map service the Source/Kilden (https://kilden.nibio.no).

4. Commissioned investigations

On the 13th of June 2019, the NIBIO laboratory received two sampling vessels (each 500 ml in volume) marked HB 168/Askøy indicating the problematic water that was taken on the 12th of June 2019 from the contaminated drinking water holding pool. The microbial analysis for the detection of *E. coli* was conducted immediately with an assigned urgent status allowing priority sample transfer to molecular QMST tests. On the 14th of June 2019, the entire investigation was completed, and a results report was delivered.

5. Additional pathogenic studies

In addition to the commissioned faecal source tracking using QMST, NIBIO took their own research initiative to study other common waterborne pathogenic bacteria in the HB 168/Askøy sample. Firstly, 100 ml of water was concentrated by ultrafiltration to collect the solid mass on membrane filter (0.45 μm). Secondly, the yielded filter was processed to extract genomic DNA using DNeasy PowerWater Kit (Qiagen GmbH, Hilden, Germany). This kit outperformed other similar products in removal of PCR inhibitors from different waters (Brandt and Albertsen, 2018; Hinlo et al., 2017). The extracted DNA purity and concentration were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, U.S.A.). Finally, TaqMan chemistry-based qPCR was performed to detect *hipO*, *16S rRNA* and *plc* specific pathogen markers for the identification of *Campylobacter jejuni* (*C. jejuni*), *Enterococcus faecalis* (*E. faecalis*) and *Clostridium perfringens* (*C. perfringens*), respectively, as well as *stx1, stx2* and *eae* specific for Shiga-toxin producing *E.coli* (*STEC*).

6. Interpretation of findings and discussion

The commissioned investigation revealed that there was faecal contamination in the drinking water HB 168/Askøy. High concentrations of coliform bacteria (>2.005E+02 MPN/100 ml) and *E. coli* (3.84E+01 MPN/100 ml) were detected. Furtherly, the microbial genomic DNA was extracted and used for the QMST analysis to determine the source of faecal water contamination. By applying the host-associated genetic markers for humans, ruminants, horses, pigs and other animals, a faecal sources distribution profile (FSDP) was depicted based on the yielded QMST results. The profile showed an exclusive (100%) zoogenic origin of faecal water contamination; thus excluded any possibility of human derived contamination, e.g. leakage from the sewage system. Moreover, the FSDP disclosed that the majority (69%) of the faecal contamination came from horses, while 6% was from ruminants and 25% from other animals (e.g. wild animals or birds).

A brief review of the worldwide reported waterborne campylobacteriosis cases shows that they were primarily caused by raw water contamination (Huang et al., 2015; Soneja et al., 2016), failures in drinking water plants (Guzman-Herrador et al., 2016; Mohammed and Seidu, 2019) or water distribution systems (Kuhn et al., 2017; Pedati et al., 2019). By studying and comparing these reports with the water health crisis in Askøy, we hypothesised a scenario in which the drinking water was polluted right before distribution. In this scenario, two possible transport routes of equine faeces into the cave with the holding drinking water pool might have been considered: 1) direct from primary vectors, e.g. horses that defecated somewhere on the hill over the holding pool and their faeces were washed down, especially after rain episodes, through the rock cracks into the pool, and 2) indirect through secondary vectors, e.g. birds and other animals scattering equine faeces or muck straight into the pool (as some openings between the cave's outer wall and ground were observed allowing penetration of the cave) or spreading them over the hill where seepages occurred.

The scenario in which *Campylobacter* spp. in equine faeces directly and/or indirectly contaminating the drinking water pool in Askøy should not be neglected since no other possible sources of the occurred epidemic have ever been identified. Multilocus sequence typing (MLST) has been frequently applied to characterise *Campylobacter* spp., with recent advancement to whole-genome sequencing (WGS) data based MLST and a core genome MLST (Cody et al., 2017). Despite the significant improvements of the detecting resolution, there are certain limitations of the WGS based approach; one of them was largely due to the incompleteness of the data obtained from the limited available clinical isolates. A study by Dearlove et al. (2016) revealed that common strains of *C. jejuni* and *C. coli*, belonging to ST-21, ST-45 and ST-828 clonal complexes could have broad host ranges, more likely due to the rapid host switch of these strains, which could impede the source attribution. Concerning these technological and intrinsic limitations, the identification, with sufficient confidence, of the genuine source of human campylobacteriosis infection in Askøy could be quite challenging.

By reviewing the previously reported campylobacteriosis outbreaks, a large number of human infectious cases were associated with the contaminated poultry (Kaakoush et al., 2015). It is well known that avian species, especially poultry, are the main natural niche for *Campylobacter* spp. (Facciolà et al., 2017). Horse originated outbreaks are rarely reported, though horses also carry *Campylobacter* spp. in the gut of either healthy or sick individuals, which can be further transmitted and infect people (Baserisalehi et al., 2007; Blunden et al., 2006; King County, 2019; Moriarty et al., 2015; OVMA, 2019). Concerning the *Campylobacter* outbreak in Askøy 2019, the horse as the dominant source of faecal water pollution might (but need not) be the cause of the epidemic. Notably, hill riding activities were observed in the surroundings, as this hilly terrain with forest and fjord landscape is quite popular for various year-round outdoor activities.

The additional laboratory studies of the problematic water detected several common waterborne pathogenic bacteria. As per 100 ml of the tested water, there were 3.44E+04 gene copies of *hipO* specific for *C. jejuni*, 9.25E+03 copies of *16S rRNA* specific for *E. faecalis* and 1.37E+05 copies of *plc* specific for *C. perfringens*, and markers for *STEC* such as *stx1* in 9.36E+03 copies, *stx2* in 8.35E+03 copies and *eae* in 5.05E+02 copies. All of them are intestinal pathogenic microbes to humans, causing gastroenteritis with the same manifested symptoms. Co-occurrence of pathogens involved in waterborne outbreaks have been reported previously, for instance in 2000 in Walkerton, Canada (Hrudey et al., 2003), in 2005 in Oregon, USA (Yoder et al., 2008) and in 2007 in Køge, Denmark (Vestergaard et al., 2007), where both *Campylobacter* and *E. coli* were found to cause the crisis. In some other occasions, *Campylobacter* together with norovirus represented the causative agents in the outbreaks in 2004 in Ohio, USA (Liang et al., 2006) and 2008 in Zurich, Switzerland (Breitenmoser et al., 2011).

7. Concluding remarks

The water health crisis in Askøy reflected all the main health threats associated with faecal water contamination, which refer to infections, illnesses and highly suspicious deaths caused by enteric pathogen(s). The identification of the infectious source is quite challenging and, in many cases, unresolved, while the determination of faecal pollution sources is more achievable. Here, we intend to highlight the possibility of applying DNA-based markers for QMST in identifying the primary faecal origin of water contamination in Askøy. The determination of the zoogenic origin considerably shortened the entire investigation to non-human (zoogenic) sources, among which horses dominated. This core information is especially useful for downstream epidemic source tracking. To this end, we recommend considering the QMST tests for systematic water quality control in order to promptly disclose any potential health threats and hence prevent eventual epidemic outbreaks.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.113420.

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