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1 **Detecting Genes Associated with Pathogenicity and Antimicrobial**

2 **Resistance in Three New Zealand Waterways.**

3

4

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26 **BACKGROUND:** More than 100 zoonoses may be transmitted via water, among them enteric
27 diseases are leading causes of human mortality. Traditional monitoring for zoonoses relies on
28 culturing of indicator species, but environmental DNA (eDNA) provides an alternative,
29 allowing direct testing for genetic loci associated with pathogenicity and/or antimicrobial
30 resistance in zoonotic bacteria.

31 **OBJECTIVES:** To evaluate whether genes associated with Shiga toxin producing *Escherichia*
32 *coli* (STEC) and antimicrobial resistance can be monitored in waterways using culture-free
33 sampling of eDNA combined with PCR-based testing.

34 **METHODS:** Water and sediment samples were collected from two sites on each of three rivers
35 in Canterbury, New Zealand; sample sites were situated above and below reaches bordered
36 by intensive dairy farming. Samples from each site were tested for genes typically associated
37 with *Escherichia coli*, STEC serogroups O26 and O157, human pathogenic strains of STEC,
38 and resistance to a broad range of antibiotics.

39 **RESULTS:** Both culturing and genetic testing confirmed the presence of *E. coli* in all samples.
40 In contrast, presence of genes associated with STEC and antibiotic resistance varied by
41 season and substrate. The O157 serogroup was identified at more than twice as many sites as
42 O26, with the latter more common in autumn samples. In autumn, genes associated with
43 pathogenic STEC were detected in one Ashley and both Rangitata River samples but were
44 present in all spring samples, except one Ashley and one Selwyn River collection. The
45 antibiotic resistance gene was only identified in spring, predominantly at sites downstream of
46 intensive dairying.

47 **DISCUSSION:** While our sample is small this study indicates that genetic testing of eDNA can
48 be a useful tool for monitoring the presence and persistence of zoonoses in waterways. How
49 the presence of these genetic elements is related to that of pathogenic STEC and incidence of
50 disease in humans now needs to be examined.

51

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52 **Introduction**

53 Globally, the degradation of freshwater ecosystems due to anthropogenic land use changes
54 has been linked to outbreaks of waterborne disease in humans (OECD 2017).
55 Industrialisation (Closs et al. 2016; Ratha 2019), urbanisation (Merritt et al. 2010; Power et
56 al. 2018), and intensive agriculture (Clapcott et al. 2012; Gluckman 2017; Joy 2015) all have
57 the potential to negatively affect freshwater ecosystems through removal of riparian
58 vegetation (Hickey and Doran 2004; Sweeney and Newbold 2014), heavy metal
59 accumulation (Hickey and Clements 1998), increased sediment and nutrient inputs (Waters
60 1995; Yan et al. 2016), and contamination with faecal effluent (Dangendorf 2004). In New
61 Zealand rapid changes in land use (Julian et al. 2017), the degradation of freshwater
62 ecosystems (Clapcott et al. 2012), and steady increases in outbreaks of notifiable bacterial
63 zoonoses are all well documented (Ministry for the Environment and Statistics New Zealand
64 2017).

65 Enteric diseases, which may be waterborne, are a leading cause of human deaths
66 worldwide, especially in children under five years of age in lower income countries (World
67 Health Organization 2019). Currently more than 100 zoonoses are recognised as being
68 transmitted in aquatic ecosystems. These include members of *Leptospira*, *Campylobacter*,
69 *Escherichia*, and *Salmonella* (Fang 2014; Gluckman 2017; Shaw et al. 2016). Human and
70 animal faecal effluent are primary sources of the microorganisms responsible for waterborne
71 zoonoses (Reddy et al. 1981). In broad terms, the risk of contracting bacterial zoonoses from
72 effluent contaminated waterways increases for those swimming in (McBride et al. 2002),
73 gathering food from (Perkins et al. 2016; Rose et al. 2001) or eating undercooked produce
74 irrigated with (Adator et al. 2018; King et al. 2012; Solomon et al. 2002) effluent
75 contaminated water. However, in specific terms, the risks associated with using effluent

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76 contaminated recreational waterways remain poorly understood because they are complex,
77 dynamic and multi-factorial (Colford Jr et al. 2007; Prieto et al. 2001).

78 Shiga toxin producing *Escherichia coli* (STEC) is an emerging group of zoonoses
79 linked to ruminants (Colford Jr et al. 2007; Oporto et al. 2019) that may occur in in
80 recreational waters and to be transmitted via the oral-faecal route (Swaggerty et al. 2018),
81 STEC is the fourth most commonly reported zoonosis in the EU (Severi et al. 2016) and USA
82 (European Center for Disease Prevention and Control 2018) and an emerging issue in New
83 Zealand (ESR 2019). In humans STEC infections typically present as enteric disease that in
84 severe cases may progress to haemolytic uraemic syndrome (HUS) or kidney failure
85 (Karmali 2018). Strains of *E. coli* recognised as belonging to the STEC group are generally
86 characterised by the presence of one or a pair of Shiga toxin producing genes (*stx1* and *stx2*)
87 (Donnenberg et al. 1997; Perna et al. 1998). However, these strains are otherwise diverse;
88 belonging to 129 serogroups and with more than 260 antigen combinations (Valilis et al.
89 2018). Typically, human pathogenic STEC strains contain the locus of enterocyte and
90 effacement (LEE) island (Paton and Paton 1998) and, until recently, were most likely to be
91 identified as belonging to the O157 serogroup (Centers for Disease Control and Prevention
92 2018; European Center for Disease Prevention and Control 2018). In New Zealand human
93 cases of STEC disease tripled between 2014 and 2017 (Health and Environment Group ESR
94 2019). However, over this same period the proportion of cases linked to the O157 serogroup
95 fell from 88.1% to 37.1% (Health and Environment Group ESR 2019). Non-O157 STECs
96 (e.g., O26, O45, O111) are increasingly being linked to disease in humans (Germinario et al.
97 2016; Gill et al. 2019; Luna-Gierke et al. 2014; Severi et al. 2016). This observation may
98 simply reflect improved screening (Health and Environment Group ESR 2019) or may be due
99 to changes in serotype prevalence.

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100 Culture for human pathogenic STEC involves the isolation of candidate organisms
101 based on their ability to metabolise different carbohydrates (Amézquita-López et al. 2018)
102 which is then followed by molecular characterisation. Molecular characterisation of STEC
103 typically focuses on genes associated with virulence (i.e., *stx1* and *stx2, eae* located on the
104 LEE island) and the serotypes most commonly found in human cases (e.g., *rfbE* for O157,
105 *wzy* for O26) (Anklam et al. 2012; Franz et al. 2007). However, in most studies of
106 environmental samples, the microbial communities are enriched, using growth media, for 18-
107 24 hours prior to plating and identification (De Boer and Heuvelink 2000; Irshad et al. 2016).
108 Enriching samples in this way is not ideal as it alters the composition of the original
109 microbial community; effects may be due to contrasting media preferences (Amagliani et al.
110 2018) or interactions between community members (Chekabab et al. 2013; Mauro et al.
111 2013). At worst such changes may result in failure to detect potentially pathogenic
112 community members.
113 Use of traditional culturing is further complicated in the case of STEC as strains may
114 lose genes associated with virulence during incubation for enrichment or culturing
115 (Senthakumaran et al. 2018; Tarr et al. 2019). An alternative to the traditional approach is to
116 test DNA extracted directly from environmental samples for the presence of genes associated
117 with virulence or antibiotic resistance (Bélanger et al. 2002; Werber et al. 2002). Testing of
118 environmental DNA (eDNA) can provide a rapid and inexpensive survey of human
119 pathogens at a site. This approach could be used to examine whether genes typically
120 associated with pathogenic STEC (e.g., *stx1*, *stx2* and *eae*) are present at a given site.
121 Although this approach does not identify pathogenic isolates, the presence of virulence genes
122 in a given sample should raise concerns about the potential level of faecal contamination at
123 that site. This approach allows us to identify sites where further investigation, including the
124 isolation and testing of individual microbes, is warranted.

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125 In this study we use a culture free, eDNA method to test for genes associated with
126 STEC from sites on three Canterbury, New Zealand rivers. Samples were collected to
127 coincide with seasonal peaks in human STEC cases during autumn and spring (Health and
128 Environment Group ESR 2019). We tested both benthic sediments and water column samples
129 for five genes frequently associated with human pathogenic STEC and the recurrently co-
130 morbid, group 1 CTX-M β-lactamases which confer resistance to multiple antibiotics (Ishii et
131 al. 2005; Valat et al. 2012). For each sample we also performed traditional *E. coli* counts to
132 determine whether there was any relationship between colony numbers and the prevalence of
133 these genes.
134

135 **Materials and Methods**

136 ***Sample Collection***

137 Water and sediment samples were collected from the Ashley, Rangitata, and Selwyn rivers
138 once in austral autumn and once in the spring of 2018. Collections were made at two sites
139 along each river; these sites were 10-15 km apart with one above and the other below reaches
140 bordered by high densities of intensive dairying (Fig.1). At each site, water and benthic
141 sediment samples were collected into separate sterile containers. Three 1 L water and three
142 25 g sediment samples were collected at each site. Samples were packed on ice, transported
143 to the laboratory, and processed within 24 hours of collection.
144

145 ***Sample Processing for Bacterial Culturing***

146 Water column sample aliquots were diluted 1:10, 1:5, and 1:2.5 with sterile MilliQ H_2O to a
147 final volume of 50 ml. At sites where the level of suspended sediments was high, an
148 additional 1:50 dilution was also prepared to ensure ease of enumeration. For each sample we
149 prepared three technical replicates at each dilution (i.e., 9-12 dilutions per sample).

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150 For sediment samples, 2 g of wet sediment was first transferred to a 5 ml microtube, 3
151 ml MilliQ H_2O added, and the mixture then agitated vigorously for 30 seconds. Aliquots of
152 the resulting supernatant were diluted 1:500, 1:200, 1:100, and 1:50 with MilliQ H_2O to a
153 final volume of 50 ml. For each sample we prepared three technical replicates at each dilution
154 (i.e., 12 dilutions per sample).
155 For each dilution the total 50 ml volume was vacuum filtered through a single sterile
156 0.45 µm cellulose ester membrane filter (Merck KGaA, Darmstadt, Germany).
157
158 ***Bacterial Culturing***
159 Bacterial culturing followed United States Environmental Protection Agency method 1603
160 (EPA 2002). Each filter was placed onto a Difco Modified mTEC Agar (VWR, Radnor, PA)
161 plate, incubated at 37.5 ^{o}C for two hours, and then incubated at 45 ^{o}C for 18-20 hours.
162 Following incubation colonies resembling *E. coli* (red/magenta colonies) were counted.
163
164 ***Sample Processing for Molecular Testing***
165 Three 500 ml aliquots of water from each site were vacuum filtered through separate 0.45 µm
166 cellulose ester membrane filters. Environmental DNA was extracted from half of each filter
167 using the NucleoSpin® soil kit (Machery-Nagel GmbH and Co. KG, Düren, Germany)
168 following the manufacturer's instructions. For each sediment sample eDNA was extracted
169 from three 0.5 g aliquots of wet sediment, using the NucleoSpin® soil kit.
170
171 ***Molecular Testing for Specific Genes***
172 The presence of five genes most commonly associated with human pathogenic STEC was
173 evaluated using a polymerase chain reaction (PCR). Specifically, we targeted genes
174 associated with serogroup specific antigen biosynthesis, *rfbE* for O157 and *wzy* for O26, the

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175 *stx*$_1$ and *stx*$_2$ toxin genes, and the intimate attachment and effacing gene, *eae*, using the
176 primers reported by Anklam et al. (2012). We also investigated the presence of antimicrobial
177 resistance by targeting the *bla* gene associated with group 1 CTX-M β-lactamases using the
178 primers reported by Lalzampuia et al. (2013). Finally, as an amplification control we targeted
179 the beta-glucuronidase gene, *uidA*, which is present in most *E. coli* (Anklam et al. 2012).
180 Amplification reactions were performed in 20 µl reaction volumes containing 0.5 × iQ
181 PerfeCTa® qPCR ToughMix™, ROX™ (QIAGEN, Düsseldorf, Germany), 1 pM of each
182 primer, and 2.5 µl of DNA template. Thermocycling was performed in a T1 thermocycler
183 (Biometra GmbH, Göttingen, Germany) using standard cycling conditions including an initial
184 denaturation at 94°C for 3 mins, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and
185 72°C for 1 min, with a final extension at 72°C for 5 mins. Amplification products were
186 visualised using SYBR Safe (ThermoFisher Scientific, Waltham, MA) following
187 electrophoresis on 2% Tris-acetate-ethylenediamine tetraacetic acid agarose gels.
188
189 **Results**
190 ***Bacterial Culturing***
191 With just one exception, the spring sampling at the above intensive dairy site on the Selwyn
192 River, *E. coli* counts were consistently higher from sediment samples than water column
193 samples (Table 1). Moreover, counts were also higher for all but one sample taken at below
194 intensive dairy sites. Only the spring sediment sample from the above intensive dairy site on
195 the Rangitata River had a higher *E. coli* count than the corresponding sample from the below
196 intensive dairy site.
197
198 ***Molecular Testing for Specific Genes***

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199 The *uidA* locus was successfully amplified from every sample in this study (Table 2). In
200 contrast, detection of the three gene loci associated with pathogenic STEC (*stx1*, *stx2* and
201 *eae*) and the gene associated with antibiotic resistance (*bla* CTX-M) varied with location,
202 time, and substrate. The *stx1*, *stx2* and *eae* genes were more frequently detected in water
203 samples in the autumn but in sediment samples in the spring. At both sampling times these
204 three virulence genes were found together with the O26 (*wzy*) and O157 (*rfbE*) genes. The
205 O26 marker was more frequently recovered from water, while that for O157 was evenly
206 distributed between the substrates. The antibiotic resistance gene was detected in both
207 substrates in samples from the Selwyn and Rangitata rivers.

208 In autumn, the genes associated with pathogenic STEC O157 (i.e., *stx1*, *stx2, eae* and
209 *rfbE*) were present at 33% of sample sites. All three virulence and both serogroup genes were
210 detected in Rangitata River water and Ashley River sediment samples from below intensive
211 dairy. Additionally, the *stx1*, *eae*, and *rfbE* genes were present in water from the site below
212 intensive dairy on the Ashley River. No other virulence or serogroup genes were detected in
213 the autumn samples.

214 In spring *stx1*, *stx2* and *eae* genes were detected in all rivers. These three STEC
215 virulence genes were detected in sediment samples from five of the six sites (i.e., all except
216 the Ashley below intensive dairy) but only from below intensive dairy in the water samples;
217 only the *eae* gene was detected in water samples from above intensive dairy. In spring
218 samples, the gene associated with the O157 serogroup (*rfbE*) was detected four times more
219 often than that associated with the O26 serogroup (*wzy*) and the *bla* CTX-M gene was more
220 frequent below intensive dairy. In three samples *stx1*, *stx2* and *eae* were detected together; in
221 one of these the gene associated with the O157 serogroup was present but the genes
222 associated with O157 and O26 were not detected in the remaining two. The *stx1* and *eae*

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223 genes were detected in two water samples from below intensive dairy whereas *stx2* and *eae*
224 were detected in three sediment samples, two from below and one above intensive dairy.
225
226 **Discussion**
227 The presence of human pathogenic STEC in New Zealand waterways is poorly documented.
228 In part, this is because STEC strains have diverse metabolic requirements; since strains may
229 co-occur, identifying individual organisms present in a sample would require multiple culture
230 methods (Kerangart et al. 2018; Possé et al. 2007). In studies on STEC strains from ruminant
231 faeces and environmental samples the microbial communities are generally enriched prior to
232 DNA-based testing (Browne et al. 2018; Irshad et al. 2016; Jaros et al. 2013). The potential
233 impacts of such enrichment on the microbial communities and on inferences concerning the
234 detection of zoonotic disease are difficult to quantify. In the present study, we tested samples
235 for genes associated with virulence and antibiotic resistance in STEC without an initial
236 enrichment step.
237 In our analyses the genetic markers indicative of *E. coli* (*uidA*) and STEC virulence
238 (*stx1*, *stx2* and *eae*) genes were detected in all three of the rivers sampled. That all samples
239 tested positive for the *uidA* gene is consistent with *E. coli* being isolated at all six sites, from
240 both substrates, and at both sampling times using traditional plating. Using relatively small
241 sample volumes we detected genes associated with both human pathogenic STEC and
242 antibiotic resistance without first enriching the microbial communities. Although all the
243 STEC virulence and O serotype genes were detected in all rivers, and in some cases all these
244 components were detected from a single sample, their presence varied by location, substrate,
245 and season. These results suggest that explanations for the distribution of these genes across
246 the landscape are likely to be complex and involve a range of factors.

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247 *Escherichia coli* levels were higher and STEC associated genes more commonly
248 detected at sites below intensive dairy. For example, the *stx1* gene was detected in six of 12
249 (50%) samples, and at least once at every site, below intensive dairy but in only two of 12
250 (17%) samples taken above intensive dairy. These results are consistent with previous reports
251 suggesting that agricultural effluent is a primary source of faecal contamination in New
252 Zealand waterways (Gluckman 2017). Studies have shown that faecal bacteria are transferred
253 from pastures to waterways via run-off; carried either directly by the flow or indirectly as a
254 result of adsorption to soil particles (Byappanahalli and Ishii 2011; Muirhead et al. 2004;
255 Palmateer et al. 1993). However, studies have suggested that paddock-feeding waterfowl may
256 also transmit ruminant hosted faecal bacteria when environmental contamination is high
257 (Yang et al. 2019; Zou et al. 2019). In the current study we sampled adjacent to intensive
258 dairy operations but expected microbial communities to vary along each river due to the
259 diversity of land uses within each catchment. Microbial communities are likely to be most
260 strongly influenced by adjoining land use but also impacted by inputs from upstream of the
261 sampling location. Moreover, microbial communities reflect the prevailing environmental
262 conditions and primary land use within the catchment. For example, the *stx1* and *stx2* genes
263 were only detected at above intensive dairy sites during spring. One explanation for this
264 observation may be inputs from smaller farms upstream of the reach bordered by the high
265 densities of intensive dairying.

266 The STEC associated genes occurred at greater frequency in the spring sampling than
267 the autumn sampling. Specifically, they were detected in eight of 12 samples (66%) in the
268 spring and in three of 12 samples (25%) in the autumn. This result reflects a combination of
269 increased detection below intensive dairy (i.e., five in September and three in May) as well as
270 detection above intensive dairy in the spring. Increased detection of STEC and antibiotic
271 resistance during the spring is likely a response to several factors. Spring calving may

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272 increase the load of pathogenic bacteria on land neighbouring these rivers. As calves have
273 poorly developed intestinal biomes, are stressed by weaning, or are removed from their
274 mother prior to receiving colostrum they are prone to colonization by and heavy shedding of
275 bacteria, including STEC (Browne et al. 2018). In addition to the increased faecal loading on
276 pastures, spring rainfall patterns may lead to higher faecal inputs reaching rivers. Other
277 possible explanations for fewer detections in the autumn include lower base flow rates or
278 drying out of rivers during the summer (e.g., the Selwyn), removal of stock from flood prone
279 areas for winter grazing, and geomorphology that encourages adsorption of bacteria to
280 sediments (Reddy et al. 1981). That STEC associated genes were more frequently detected
281 during the spring is in contrast to the seasonal cycle of human STEC disease cases in New
282 Zealand. Although there is a peak in notified human cases during spring, the autumn peak is
283 typically larger. For example, in 2017 there were almost twice as many cases of STEC in
284 autumn than during spring (Health and Environment Group ESR 2019). Moreover, although
285 rates of notified human cases of STEC do differ between regions the differences are not
286 always consistent with the prevalence of dairy farming. For example, in 2017 the Auckland
287 and Waikato districts reported 7.4 and 8.6 cases per 100,000 individuals despite dairy
288 farming being far more common in the Waikato region (Health and Environment Group ESR
289 2019). Taken together these observations suggest that livestock are unlikely to be the sole
290 source of human STEC infections in New Zealand and that we need to be cautious when
291 interpreting data on the distribution of STEC, or the genetic components linked to it, in the
292 context of public health.

293 In all three of the sampled rivers *E. coli* levels were consistently higher in sediments
294 than in the water column. The STEC associated genes were also more commonly detected in
295 sediment samples. These results are consistent with previous studies that indicate aquatic
296 sediments may act as a store for *E. coli* (Muirhead et al. 2004; Perkins et al. 2014; Wilkinson

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297 et al. 1995). Given that such stores may persist for months or years (Anderson et al. 2005;
298 Garzio-Hadzick et al. 2010; Gerba and McLeod 1976) and that suspended sediments increase
299 *E. coli* levels in the water column (Davies-Colley et al. 2018; Weiskerger and Whitman
300 2018) this finding has potentially important implications for water monitoring. In New
301 Zealand, water quality testing by local government agencies is currently restricted to the
302 water column. Although recreational use of waterways is discouraged when levels of
303 suspended sediment are high (e.g., following precipitation) (Davies-Colley et al. 2018),
304 disturbance of sediments by recreational users is not generally considered. Further work is
305 needed to quantify the pathogens associated with the localised mixing of sediment into the
306 water column by recreational users and to determine whether zoonoses are being
307 underestimated by the sampling of a single substrate.
308
309 **Conclusion**
310 Microbial culturing is not an efficient tool for monitoring STEC in recreational waterways. In
311 part this can be attributed to the metabolic diversity of STEC and the apparent lack of a
312 relationship between faecal indicator bacteria (e.g., *E. coli* counts) and the presence of genes
313 associated with STEC. Understanding this relationship would require more intensive
314 sampling over a broader geographic range. In most cases monitoring is conducted on
315 samples retrieved from the water column. Such samples may not accurately reflect the
316 microbial community that recreational users of the waterway may be exposed to. Our results
317 suggest sediments may act as an important STEC reservoir and resuspension of these
318 sediments by waterway users could potentially increase exposure to STEC.
319 There is a growing appreciation of new technologies that enable the presence and
320 persistence of zoonoses to be monitored without the need for microbial culturing
321 (Byappanahalli and Ishii 2011; Rose et al. 2001). This represents a fundamental change in

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322 our approach to microbial monitoring allowing us to take a holistic view of the riverine
323 environment and improving our ability to ensure environmental, animal, and human health.
324 While small, this study is the first step towards understanding zoonoses in New Zealand
325 waterways at a time when global health is under the microscope.

326

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330

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Table 1. *Escherichia coli* colony counts, in CFU/100 ml, for water and sediment samples collected from the Selwyn, Rangitata, and Ashley rivers during May and September 2018.

Sampling time	Sampling sites and substrates											
	Above intensive dairy						Below intensive dairy					
	Ashley water	Ashley sediment	Rangitata water	Rangitata sediment	Selwyn water	Selwyn sediment	Ashley water	Ashley sediment	Rangitata water	Rangitata sediment	Selwyn water	Selwyn sediment
May (autumn)	45	230	20	7,100	10	200	440	730	175	273,300	160	230
September (spring)	20	400	20	5,000	505	200	2,000	3,600	40	1,600	2,250	12,200

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Table 2. Presence of *Escherichia coli* control (*uidA*), STEC virulence (*stx1*, *stx2*, *eae*), serogroup (O26 *wzy* and O157 *rfbE*), and antibiotic resistance (*bla* CTX-M) genes in water and sediment samples collected from the Selwyn, Rangitata, and Ashley rivers during May and September 2018.

Gene locus	Sampling sites and substrates											
	Above intensive dairy						Below intensive dairy					
	Ashley water	Ashley sediment	Rangitata water	Rangitata sediment	Selwyn water	Selwyn sediment	Ashley water	Ashley sediment	Rangitata water	Rangitata sediment	Selwyn water	Selwyn sediment
May (autumn)												
uidA	+	+	+	+	+	+	+	+	+	+	+	+
stx1	–	–	–	–	–	–	+	+	+	–	–	–
stx2	–	–	–	–	–	–	–	+	+	–	–	–
eae	–	–	–	–	–	–	+	+	+	–	–	–
wzy O26	–	–	–	–	–	–	+	+	+	–	–	–
rfbE O157	–	–	–	–	–	+	+	+	+	–	–	+

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bla CTX-M	–	–	–	–	–	–	–	–	–	–	–	–
September (spring)												
uidA	+	+	+	+	+	+	+	+	+	+	+	+
stx1	–	+	–	+	–	–	–	–	+	–	+	+
stx2	–	+	–	+	–	+	+	–	–	+	–	+
eae	–	+	+	+	–	+	+	+	+	+	+	+
wzy 026	–	–	–	–	–	–	–	–	+	–	–	–
rfbE O157	–	–	–	–	–	+	–	–	–	+	+	+
bla CTX-M	–	–	–	+	–	–	–	–	+	–	+	+

604 + Gene detected

605 – Gene not detected

606

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607 **Figure Legends**

608

609 **Figure 1.** Map of central and southern Canterbury, New Zealand with the Ashley, Rangitata,

610 and Selwyn rivers labeled. Locations at which water and sediment were collected during May

611 and September 2018 are marked with circles; for each river the white centered dot indicates

612 the above intensive dairy site and the black centered dot the below intensive dairy site.

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