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1 Short Communication

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3 **A *Cryptosporidium parvum* genotype shift between week old and**
4 **two week old calves following administration of a prophylactic**
5 **antiprotozoal.**

6

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28 **Abstract**

29 This study looked to assess the stability of *Cryptosporidium parvum* genotypes in calves between the
30 final day of treatment with the antiprotozoal halofuginone lactate and seven days post-treatment. Paired
31 faecal samples were collected on the final day of treatment and seven days later from 54 calves across
32 seven farms in South-west England. The presence of *Cryptosporidium* species was detected using
33 polymerase chain reaction targeting the 18s rDNA. The presence and genotype of *C. parvum* was
34 determined by PCR and amplicon sequencing targeting the gp60 locus. On farms where *C. parvum*
35 was detected at both sampling times there was a distinct genotype shift. Detection of gp60 genotype
36 IIaA15G2R1 decreased from 40% to 7% while IIaA17G1R1 increased from 0% to 41%, supplemented
37 by IIaA16G3R1 in one sample. A shift in *C. parvum* genotypes present in calves within a one week
38 sampling timeframe has not been described prior to this study, indicating that the timeframe is likely
39 suitable for observing variation in *C. parvum* populations and interactions with antiprotozoal control
40 strategies.

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44 **Keywords:** *Cryptosporidium parvum*, halofuginone lactate, calves, genotype, gp60.

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48 **Highlights**

- 49 • A single *Cryptosporidium parvum* genotype was initially dominant on multiple farms
- 50 • gp60 genotypes became more diverse within a one week sampling timeframe
- 51 • First description of a *C. parvum* genotype shift in neonate calves
- 52 • Explanatory factors considered: drug interaction and maturation of calves

53

54 **1. Introduction**

55 *Cryptosporidium parvum* is a zoonotic parasite prevalent among UK calves, reported to occur at levels
56 of 45.1% in individuals under one month of age (Smith et al., 2014). Infection largely results in morbidity
57 in juveniles, associated with diarrhoea, anorexia and dehydration, although for immunocompromised
58 individuals further complications can arise (Taylor et al., 2007, De Graaf et al., 1999). Halofuginone
59 lactate is licensed for control and treatment of *C. parvum* in UK cattle; however the effects of treatment
60 on *C. parvum* population structure has not been defined.

61

62 *Cryptosporidium* follows a lifecycle broadly in line with other members of the suborder Eimeriorina with
63 three key phases: sporulation, schizogony, and gametogony. Notably, sporulation occurs within the
64 host and not externally, meaning oocysts are capable of auto-infection of the same host as well as
65 being immediately infectious upon shedding (Taylor et al., 2007). Following the initiation of infection it
66 takes a minimum 72 hours for progeny oocysts to be produced (the pre-patent period; (Taylor et al.,
67 2007)); with self-limiting infections lasting around two weeks (Olson et al., 1999). The tough oocyst wall
68 permits significant longevity in the environment. Overall, oocysts can survive in soil and faecal matter
69 for up to 12 weeks at below 25°C, and for over 12 weeks in water (Olson et al., 1999), making effective
70 control and biosecurity a challenge for farmers. One option for control is the use of halofuginone lactate,
71 licensed for treatment of *Cryptosporidium* in calves for both prophylactic (prevention of diarrhoea) and
72 therapeutic purposes (reduction of diarrhoea). It is most commonly used prophylactically and is
73 administered for seven days orally to calves starting from 24-48 hours old (European Medicines Agency,
74 2016). Field and trial studies have shown efficacy in reducing *Cryptosporidium* oocyst shedding when
75 investigating faecal presence compared to placebo treatment. Commonly, data has been collected from
76 the final day of treatment (~ 7-9 days) and thereafter at regular intervals up to ~28 days.

77

78 Jarvie and colleagues showed that, in the first month of life, halofuginone treated calves excreted 70%
79 fewer oocysts than placebo treated calves ($p < 0.05$) (Jarvie et al., 2005); and Lefay observed that calves
80 after seven days of halofuginone treatment excreted 44% fewer oocysts compared to a placebo
81 ($p < 0.05$) (Lefay et al., 2001). Conversely, Almawly observed only a delay in oocyst shedding by
82 halofuginone treated calves but no overall significant difference in total numbers or the occurrence of
83 diarrhoea (Almawly et al., 2013). Trotz-Williams noted a difference only in overall oocyst shedding, not
84 in the occurrence of diarrhoea or body weight gain (Trotz-Williams et al., 2011). Finally, De Waele's
85 study noted halofuginone was more successful (reduction in oocyst excretion and diarrhoea) when high-
86 level farm hygiene was also present (De Waele et al., 2010). Overall, halofuginone is considered to
87 reduce oocyst shedding and mitigate against diarrhoea and weight loss.

88

89 Infection and oocyst shedding is expected to resume post-treatment (Trotz-Williams et al., 2011, Jarvie
90 et al., 2005) but, considering Zambriski's work, it is still beneficial to treat since the onset of shedding
91 can be delayed and the overall yield reduced (Zambriski et al., 2013). Delaying the onset of oocyst
92 shedding can reduce environmental contamination, decrease the severity of subsequent disease and
93 the consequential economic burden. While the influence of parasite occurrence has been considered,
94 the impact of genetic variation within *C. parvum* populations has not been assessed. For example, the
95 efficacy of halofuginone for treatment of *C. parvum* in calves may be influenced by underlying genetic
96 variation and parasite population structure. *C. parvum* has historically been distinguished from other
97 parasite species morphologically and later by zoonotic capacity. Now, the accessibility of molecular-
98 based discrimination has enabled accurate sub-species genotyping based on markers such as the gp60
99 coding sequence (Chako et al., 2010). This study aimed to define variation in *C. parvum* genotype
100 occurrence using the sampling timeframe of one to two weeks as determined in previous studies of
101 halofuginone efficacy. The hypothesis followed that the diversity of *C. parvum* genotypes in calf faecal
102 samples would vary between the final day of treatment with the antiprotozoal halofuginone and seven
103 days post-treatment.

104

105 **2. Materials and methods**

106 *2.1 Sampling*

107 To evaluate temporal genetic variation in *C. parvum* paired faecal samples were required from calves.
108 The differential excretion of *Cryptosporidium* oocysts at the final day of halofuginone treatment and
109 seven days post-treatment has previously been used to indicate treatment efficacy, as observed in the
110 studies referenced above. On this basis, the same sampling timeframe was adopted to assess variation
111 in genotype occurrence. Sampling before treatment was not viable since neonatal prophylaxis
112 commonly begins at 24/48 hours old (European Medicines Agency, 2003), while oocyst shedding
113 begins a minimum of 72 hours after infection (the pre-patent period; (Taylor et al., 2007)).

114

115 Sampling packs were distributed to farms in Dorset, UK, between April 26th and June 1st, 2018. Sample
116 packs consisted of: 50ml Falcon polypropylene tubes preloaded with 10ml of 2.5% (w/v) potassium
117 dichromate to prevent bacterial proliferation and degradation of any oocysts (Olson et al., 1999),
118 instructions, farm information questionnaire and consent form. The questionnaire was designed to
119 identify herd size and breed, type of production system, and history of halofuginone use. Ethical review
120 was undertaken by the Royal Veterinary College Clinical Research Ethical Review Board and approved
121 under reference M2017 0124.

122

123 Farmers non-invasively collected approximately 20ml faeces during voiding at the final day of
124 prophylactic halofuginone treatment and seven days post-treatment. Calf ID, age and collection date
125 were all recorded. Samples were stored at 4 °C and then returned to the farms' veterinary practice and
126 thence to the Royal Veterinary College (RVC) within two weeks. The questionnaire recorded the
127 management system adopted by each farm. The questionnaire and instructions were tested by the RVC
128 farm administrator for clarity prior to application.

129

130 *2.2 Sample Processing*

131 Faecal samples were analysed on receipt in no specific order. A modified protocol for a QIAamp Fast
132 DNA Stool Extraction Kit (Qiagen, Hilden, Germany) was used to extract whole genomic DNA (gDNA)
133 from *Cryptosporidium* present in faecal samples. Briefly, a faecal sample was mixed and sub-sampled
134 for ~0.2 g solid matter, centrifuged at 10,000 g for 1 minute and the supernatant discarded, leaving the
135 pellet. Each sample was combined with glass beads (0.4 - 0.6 mm; Sigma, UK) equal to 0.5× the pellet's
136 volume and 1 ml of InhibitEX buffer (Qiagen, Hilden, Germany), and homogenised using a BeadBeater

137 at 3,000 × oscillations/min for 1 minute. The rest of the protocol followed the manufacturer's instructions.
138 Samples were stored at -20 °C prior to PCR.

139

140 2.3 PCR and Gel Electrophoresis

141 Diagnostic nested PCR targeting a fragment of the *Cryptosporidium* 18S rRNA was carried out using
142 gDNA with the primers 5'-GGAAGGGTTGTATTTATTAGATAAAG-3' (forward) and 5'-
143 AGGAGTAAGGAACAACCTCCA-3' (reverse) in the first round (Xiao et al., 19990, and 5'-
144 AGTGACAAGAAATAACAATACAGG-3' (forward) and 5'-CCTGCTTTAAGCACTCTAATTTTC-3'
145 (reverse) in the second (Abe et al., 2002), employing reagents and conditions as described previously
146 (Nolan et al., 2017). Subsequently, all samples that had been positive for *Cryptosporidium* in the 18S
147 rRNA assay were also subjected to PCR targeting a fragment of the 60 kDa glycoprotein (gp60) coding
148 sequence using the primers 5'-ATAGTCTCCGCTGTATTC-3' (forward) and 5'-
149 GGAAGGAACGATGTATCT-3' (reverse) as described previously (Nolan et al., 2017). Amplicons were
150 resolved using a 1.5% (w/v) agarose (ThermoFisher Scientific, Hemel Hempstead, UK) gel made with
151 0.5× TBE and stained with 0.01% (v/v) SafeView Nucleic Acid Stain (Novel Biological Solutions,
152 Huntingdon, UK). Electrophoresis was carried out at 40 V for 40 mins in 0.5× TBE buffer.

153

154 2.4 Sequencing

155 Amplicons for gp60 from all putatively *Cryptosporidium* positive samples were purified using a MinElute
156 PCR Purification Kit (Qiagen, Hilden, Germany) following the manufacturer instructions. Concentrations
157 were standardised to 30 ng/μl using a spectrophotometer (DeNovix, Wilmington, USA) and then
158 sequenced using the primers employed in the original reaction by GATC Biotech (GATC Biotech,
159 Cologne, Germany). Sequences were assembled against the reference KY499051 (Genbank®) with
160 default parameters on CLC Main Workbench v6.9.1 (CLC bio, Aarhus, Denmark) and gp60 genotypes
161 annotated to confirm the presence of *C. parvum* following the conventional nomenclature (Xiao, 2010).
162 All sequences have been deposited with GenBank under the accession numbers LR594827-LR594829.

163

164 2.5 Statistical Analysis

165 Analysis was carried out using IBM SPSS Statistics version 25. The significance of variation in paired
166 genotype occurrence was assessed between days 7 and 14 for no genotype present or genotypes

Abbreviations: Halofuginone lactate: Halofuginone, 60 kDa glycoprotein: gp60

167 IlaA15G2R1, IlaA17G1R1 or IlaA16G3R1 using the McNemar test. Results were considered to be
168 significant when $p < 0.05$. Additionally, the Kappa statistic of agreement was calculated, testing whether
169 the results were in agreement between the two sampling days.

170

171 **3. Results**

172 The sample size comprised 54 calves from seven farms in South-west England, including between 5
173 and 10 calves per farm (Table 1). All farms were commercial dairy systems, reported a herd size of
174 over 100 head and either had Holstein or Holstein-Friesian cattle. All farms tested routinely used
175 halofuginone prophylactically and, at a minimum, had been using halofuginone for two years.

176

177 Sampling on the final day of halofuginone treatment found three of the seven farms tested to include
178 calves positive for *Cryptosporidium* based upon a positive 18S rRNA PCR (43%; Table 1). Between
179 two and seven calves were positive on each farm. Repeat sampling seven days post-treatment detected
180 *Cryptosporidium* on all farms (100%), with two to six calves positive per farm. It should be noted that
181 the PCR test used was qualitative, and the level of *Cryptosporidium* excretion was not determined
182 per individual. Targeted sequencing of a *gp60* fragment revealed a total of three *C. parvum gp60*
183 genotypes, all of which had been described previously (Smith et al., 2014). IlaA15G2R1 was the only
184 *gp60* genotype detected at the conclusion of halofuginone treatment (Table 1). Sampling seven days
185 later identified IlaA15G2R1, IlaA17G1R1 and IlaA16G3R1 *gp60* genotypes; including four farms that
186 were host to more than one genotype.

187

188 Pairwise comparison of *Cryptosporidium gp60* genotype occurrence between sampling days 7 and 14
189 using the McNemar test was not statistically significant, likely influenced by the low sample size.
190 However, comparison using the Kappa statistic produced a Kappa value of 0.136 (standard error 0.076),
191 suggesting that the strength of association was 'poor' and indicating notable variation. By considering
192 paired samples at an individual calf level (Table 2), it was possible to differentiate persistent and
193 apparently varied infections.

194

195 **4. Discussion**

196 This study aimed to define the occurrence of *C. parvum* genotypes immediately following seven days
197 routine halofuginone prophylaxis and one week post-treatment. The results showed that the single
198 subtype IlaA15G2R1 was initially dominant on multiple farms in Dorset, UK, and that over the short
199 experimental timeframe genotypes became identifiably more diverse with the emergence of
200 IlaA17G1R1 and IlaA16G3R1. It is not possible to conclusively determine the cause of the change in
201 genotype complexity, however likely reasons can be explored. The first consideration is that the shift
202 was a result of the drug treatment. In the absence of a functional association between gp60 and the
203 outcome of halofuginone treatment, the genotype was used as a genetic marker. A genotype change
204 during the seven days after halofuginone withdrawal might suggest greater resistance defined
205 IlaA15G2R1, and greater susceptibility IlaA17G1R1, although it is possible that drug dosing was
206 inefficient. Unfortunately, a no-treatment control was not available to this study so causality cannot be
207 concluded. Despite extensive effort through the veterinary practice associated with the study, and
208 others in the region, we were unable to identify farmers who did not routinely medicate their calves.
209 Asking farmers to stop medicating their calves was not considered on ethical grounds.

210

211 Alternatively, the change in *C. parvum* genotypes could have been associated with calf maturation
212 rather than drug treatment. This may include an age-associated infection where, as calves become
213 more immunocompetent with increasing age, they become more resistant to certain genotypes.
214 Previous studies have suggested that *C. parvum* genotypes remain stable between week old and 2-4
215 week old calves in the absence of halofuginone prophylaxis (Thomson, 2015). It is also possible that
216 one or more calves may have moved from their pens during the experimental period and become
217 exposed to *C. parvum* genotypes in other environments, although no movement was reported.
218 Irrespective of the reason for the early dominance of IlaA15G2R1; the expansion of genotype
219 complexity within a short timeframe could provide an explanation why farms experience repeated bouts
220 of *C. parvum* infections; with immunity only covering individual genotypes. Notably, multiple farms have
221 shown the same pattern of early dominance by IlaA15G2R1, followed by the emergence of
222 IlaA17G1R1. This might be linked to geo-regional clustering which is common with *C. parvum*
223 genotypes (Brook et al., 2009) and might be associated with wildlife, livestock, human and/or machinery
224 movements.

225

226 **5. Conclusion**

227 This study indicates that the collection of paired samples from one and two week old calves is
228 appropriate to detect variation in *C. parvum* genotypes with relevance to the assessment of prophylactic
229 drug efficacy. A distinct genotype shift was recorded in neonatal calves for the first time, with possible
230 drivers including drug resistance or host maturation. Evidence for geo-regional clustering and repeat
231 bouts of infection have also been considered. Further research is needed to analyse genotype
232 association with the outcome of halofuginone treatment in a larger sample set, including a broader
233 genome wide genetic analysis with additional sampling points during and after treatment.

234

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241

242 Declaration of interest: None

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246 **References**

- 247 Abe, N., Sawano, Y., Yamada, K., Kimata, I., Iseki, M., 2002. Cryptosporidium infection in dogs in
248 Osaka, Japan. *Vet Parasitol* 108, 185-193.
- 249 Almajali, J., Prattley, D., French, N. P., Lopez-Villalobos, N., Hedgespeth, B. and Grinberg, A. (2013)
250 'Utility of halofuginone lactate for the prevention of natural cryptosporidiosis of calves, in the
251 presence of co-infection with rotavirus and Salmonella Typhimurium', *Veterinary Parasitology*,
252 197(1-2), pp. 59-67.
- 253 Brook, E. J., Anthony Hart, C., French, N. P. and Christley, R. M. (2009) 'Molecular epidemiology of
254 Cryptosporidium subtypes in cattle in England', *The Veterinary Journal*, 179(3), pp. 378-82.
- 255 Chako, C. Z., Tyler, J. W., Schultz, L. G., Chiguma, L. and Beerntsen, B. T. (2010) 'Cryptosporidiosis
256 in People: It's Not Just About the Cows', *Journal of Veterinary Internal Medicine*, 24(1), pp.
257 37-43.
- 258 De Graaf, D. C., Vanopdenbosch, E., Ortega-Mora, L. M., Abbassi, H. and Peeters, J. E. (1999) 'A
259 review of the importance of cryptosporidiosis in farm animals', *International Journal for*
260 *Parasitology*, 29(8), pp. 1269-87.
- 261 De Waele, V., Speybroeck, N., Berkvens, D., Mulcahy, G. and Murphy, T. M. (2010) 'Control of
262 cryptosporidiosis in neonatal calves: Use of halofuginone lactate in two different calf rearing
263 systems', *Preventive Veterinary Medicine*, 96(3-4), pp. 143-151.
- 264 European Medicines Agency (2003) *Scientific Discussion - Halocur*. Available at:
265 [http://www.ema.europa.eu/docs/en_GB/document_library/EPAR -](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/veterinary/000040/WC500064770.pdf)
266 [_Scientific_Discussion/veterinary/000040/WC500064770.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/veterinary/000040/WC500064770.pdf).
- 267 European Medicines Agency (2016) *Annex I - Summary of product characteristics*. Available at:
268 [http://www.ema.europa.eu/docs/en_GB/document_library/EPAR -](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/004201/WC500219134.pdf)
269 [_Product_Information/veterinary/004201/WC500219134.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/004201/WC500219134.pdf) (Accessed: 09/08/2018).
- 270 Jarvie, B. D., Trotz-Williams, L. A., McKnight, D. R., Leslie, K. E., Wallace, M. M., Todd, C. G.,
271 Sharpe, P. H. and Peregrine, A. S. (2005) 'Effect of halofuginone lactate on the occurrence of
272 Cryptosporidium parvum and growth of neonatal dairy calves', *Journal of Dairy Science*,
273 88(5), pp. 1801-6.
- 274 Lefay, D., Naciri, P., Poirier, R. and Chermetter, R. (2001) 'Efficacy of halofuginone lactate in the
275 prevention of cryptosporidiosis in suckling calves', *Veterinary Record*, 148(4), pp. 108-112.

- 276 Nolan, M. J., Unger, M., Yeap, Y. T., Rogers, E., Millet, I., Harman, K., Fox, M., Kalema-Zikusoka, G.
277 and Blake, D. P. (2017) 'Molecular characterisation of protist parasites in human-habituated
278 mountain gorillas (*Gorilla beringei beringei*), humans and livestock, from Bwindi impenetrable
279 National Park, Uganda', *Parasites and Vectors*, 10(1), pp. 340.
- 280 Olson, M., Goh, J., Phillips, M., Guselle, N. and McAllister, T. (1999) 'Giardia Cyst and
281 *Cryptosporidium* Oocyst Survival in Water, Soil, and Cattle Feces', *Journal of Environmental*
282 *Quality*, 28(6), pp. 1991-1996.
- 283 Smith, R. P., Clifton-Hadley, F. A., Cheney, T. and Giles, M. (2014) 'Prevalence and molecular typing
284 of *Cryptosporidium* in dairy cattle in England and Wales and examination of potential on-farm
285 transmission routes', *Veterinary Parasitology*, 204(3-4), pp. 111-9.
- 286 Taylor, M. A., Coop, R. L. and Wall, R. (2007) *Veterinary parasitology*. 3rd ed. edn. Oxford: Blackwell.
- 287 Thomson, S. (2015) *Cryptosporidiosis in farm livestock* Student Final Report No. 7785). Available at:
288 [http://beefandlamb.ahdb.org.uk/wp-content/uploads/2013/04/7785-Sarah-Thomson-Final-](http://beefandlamb.ahdb.org.uk/wp-content/uploads/2013/04/7785-Sarah-Thomson-Final-Project-Report-261015.pdf)
289 [Project-Report-261015.pdf](http://beefandlamb.ahdb.org.uk/wp-content/uploads/2013/04/7785-Sarah-Thomson-Final-Project-Report-261015.pdf).
- 290 Trotz-Williams, L. A., Jarvie, B. D., Peregrine, A. S., Duffield, T. F. and Leslie, K. E. (2011) 'Efficacy of
291 halofuginone lactate in the prevention of cryptosporidiosis in dairy calves', *Veterinary Record*,
292 168(19), pp. 5.
- 293 Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A.A., Montali, R.J., Fayer, R., Lal, A.A.,
294 1999. Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA
295 gene locus. *Appl Environ Microbiol* 65, 1578-1583.
- 296 Xiao, L. (2010) 'Molecular epidemiology of cryptosporidiosis: an update', *Exp Parasitol*, 124(1), pp. 80-
297 9.
- 298 Zambriski, J. A., Nydam, D. V., Bowman, D. D., Bellosa, M. L., Burton, A. J., Linden, T. C., Liotta, J.
299 L., Ollivett, T. L., Tondello-Martins, L. and Mohammed, H. O. (2013) 'Description of fecal
300 shedding of *Cryptosporidium parvum* oocysts in experimentally challenged dairy calves',
301 *Parasitology Research*, 112(3), pp. 1247-1254.

302

303

304 Tables

305 **Table 1.** Summary of *Cryptosporidium parvum* genotypes detected in one and two week old calves.

306 The numbers in brackets indicate the number of calf samples submitted per farm.

<i>gp60</i> genotype	Farm	Number of calves positive for <i>C. parvum</i> (total calves)	
		Final day of treatment	7 days post-treatment
IIaA15G2R1	A	0 (8)	2(7)
	B	3(10)	1(9)
	C	7(10)	1(10)
	D	0(6)	0(6)
	E	0(5)	2(5)
	F	0(5)	0(5)
	G	2(10)	0(10)
	Total	12 (54)	5 (52)
IIaA17G1R1	A	0(8)	0(7)
	B	0(10)	5(9)
	C	0(10)	3(10)
	D	0(6)	2(6)
	E	0(5)	2(5)
	F	0(5)	1(5)
	G	0(10)	4(10)
	Total	0 (54)	16 (52)
IIaA16G3R1	G	0(10)	1(10)
	Total	0 (54)	1 (52)

307

308

309

310 **Table 2.** *gp60* genotypes for calves found to be *Cryptosporidium* positive at the conclusion of
311 halofuginone treatment and seven days later. Calves positive at a single time point are not shown.

Farm	Calf ID	Final day of treatment	7 days post-treatment
B	2.7	IlaA15G2R1	IlaA17G1R1
B	2.8	IlaA15G2R1	IlaA15G2R1
C	3.2	IlaA15G2R1	IlaA15G2R1
C	3.4	IlaA15G2R1	IlaA17G1R1
C	3.8	IlaA15G2R1	IlaA17G1R1
G	7.9	IlaA15G2R1	IlaA17G1R1

312