1 **Reduced antibacterial drug resistance and *bla*_{CTX-M} β -lactamase gene carriage**
2 **in cattle-associated *Escherichia coli* at low temperatures, at sites dominated**
3 **by older animals and on pastureland: implications for surveillance.**

4

5 **Hannah Schubert¹, Katy Morley¹, Emma F. Puddy^{1,2}, Robert Arbon³, Jacqueline**
6 **Findlay², Oliver Mounsey², Virginia C. Gould^{1,2}, Lucy Vass^{1,4}, Madeleine Evans¹,**
7 **Gwen M. Rees¹, David C. Barrett¹, Katy M. Turner¹, Tristan A. Cogan¹, Matthew**
8 **B. Avison^{2*}, Kristen K. Reyher¹**

9

- 10 1) Bristol Veterinary School, University of Bristol
11 2) School of Cellular and Molecular Medicine, University of Bristol
12 3) Jean Golding Institute, University of Bristol
13 4) Bristol Population Health Science Institute, University of Bristol

14 * Corresponding author: bimba@bristol.ac.uk

15

16 Running title: Resistance in cattle-associated *E. coli*

17 **Abstract**

18 Little is known about the drivers of critically important antibacterial resistance in
 19 species with zoonotic potential present on farms (e.g. CTX-M β -lactamase-positive
 20 *Escherichia coli*). Here, we collected samples, monthly over a two-year period, on 53
 21 dairy farms in the South West of England, and data for 610 variables concerning
 22 antimicrobial usage, management practices and meteorological factors. We detected
 23 *E. coli* resistant to amoxicillin, ciprofloxacin streptomycin and tetracycline,
 24 respectively, in 2754/4145 (66%), 263/4145 (6%), 1475/4145 (36%) and 2874/4145
 25 (69%) of all samples from faecally contaminated sites. *E. coli* positive for *bla*_{CTX-M}
 26 were detected in 224/4145 (5.4%) of samples. Multilevel, multivariable logistic
 27 regression showed antibiotic dry cow therapeutic choice (including use of
 28 cefquinome or framycetin) to be associated with increased odds of *bla*_{CTX-M} positivity.
 29 Low temperature was associated with reduced odds of *bla*_{CTX-M} *E. coli* positivity in
 30 samples and to reduced odds of finding *E. coli* resistant to each of the four test
 31 antibacterials. This was additional to the effect of temperature on total *E. coli* density.
 32 Furthermore, samples collected close to calves had increased odds of having *E. coli*
 33 resistance to each antibacterial or positive for *bla*_{CTX-M}. Samples collected on
 34 pastureland had reduced odds of having *E. coli* resistant to amoxicillin or
 35 tetracycline, and being positive for *bla*_{CTX-M}.

36 **Importance**

37 Antibacterial resistance poses a significant threat to human and animal health and
 38 global food security. Surveillance for resistance on farms is important for many
 39 reasons, including to track the impacts of interventions aimed at reducing the
 40 prevalence of resistance. In this epidemiological survey of dairy farm antibacterial

41 resistance, we show that local temperature, as it changes over the course of a year,
 42 is associated with the prevalence of antibacterial resistant *E. coli*. Also, that
 43 prevalence of resistant *E. coli* is higher in indoor environments and in environments
 44 inhabited by young animals. These findings have profound implications for routine
 45 surveillance and for surveys carried out for research. They provide important
 46 evidence that sampling at a single time-point and/or single location on a farm is
 47 unlikely to be adequate to accurately determine the status of the farm with regard to
 48 the presence or number of resistant *E. coli*.

49 **Introduction**

50 Antimicrobial resistance - and particularly antibacterial resistance (ABR) - is a
 51 significant global challenge. Many countries are implementing plans to reduce the
 52 use of antibacterial drugs (ABs) in food-producing animals. For example, the most
 53 recent UK five-year National Action Plan includes a target to reduce AB use (ABU) in
 54 the treatment of food-producing animals by 25% (1). In Europe, AB sales for food-
 55 producing animals fell by 20% from 2011 to 2016 (2). In the UK dairy industry,
 56 overall ABU dropped from 24 mg/kg in 2015 to 17 mg/kg in 2018 (3, 4). In 2018,
 57 additional industry-led policies were enforced in the UK that aimed to almost
 58 eliminate the use of highest-priority critically important antimicrobials (HP-CIAs) such
 59 as third- and fourth-generation cephalosporins (3GCs and 4GCs) as well as
 60 fluoroquinolones on dairy farms. One reason for reducing ABU in farming is to
 61 reduce the prevalence of ABR bacteria carried by farm animals. However, there is a
 62 need for better data on drivers of ABR in farming. More granularity of understanding
 63 is required concerning the risks of using individual ABs and other management
 64 practices. This is especially important in terms of drivers of HP-CIA resistance. A
 65 focus among HP-CIAs is on 3GC and fluoroquinolone resistance in *Escherichia coli*,
 66 a species commonly found in animal faeces and considered one of the most
 67 significant potential zoonotic threats to humans (5).

68 3GC resistance is increasingly prevalent in *E. coli* causing infections in humans (6)
 69 and is also found in farmed and domestic animals around the world (7). The
 70 production of CTX-M (an extended-spectrum β -lactamase) is the most common
 71 mechanism of 3GC resistance in *E. coli* in humans in the UK; for example, in a
 72 recent study of urinary *E. coli* from humans in South West England, 82.2% of 3GC-
 73 resistant isolates carried *bla*_{CTX-M} (8).

74 The objective of this study was to describe the prevalence of 3GC-resistant *E. coli*
 75 carrying *bla*_{CTX-M} as well as *E. coli* resistant to amoxicillin, tetracycline, streptomycin
 76 and the fluoroquinolone ciprofloxacin found in faecally contaminated environments of
 77 dairy cattle in a geographically restricted population of dairy farms in South West
 78 England. Furthermore, this study investigated environmental, ABU and management
 79 practice risk factors for the presence of such *E. coli*.

80

81 **Results**

82 **Prevalence and PCR characterisation of 3GC-resistant *E. coli* from dairy farms**

83 4581 samples from faecally contaminated sites were collected from 53 dairy farms.
 84 Samples were collected on each farm monthly between January 2017 and
 85 December 2018. 4145 samples were positive for growth of *E. coli* on non-selective
 86 agar. Of these, 384/4145 (9.3%) samples representing 47/53 (88.7%) of farms were
 87 positive for growth of *E. coli* on agar containing the 3GC cefotaxime. From these,
 88 1226 3GC-resistant isolates were taken forward for PCR testing for possible
 89 cephalosporinase genes of interest (GOIs): *bla*_{CTX-M} (groups 1, 2, 8, 9 and 25),
 90 *bla*_{CMY}, *bla*_{DHA} and *bla*_{SHV}. Over half (648/1226; 52.7%) of all isolates tested were
 91 found to harbour *bla*_{CTX-M} genes. Of these, 547/648 (84.4%) were of group 1, 99/648
 92 (15.3%) were of group 9, and, in one case, both gene groups were identified. Twelve
 93 isolates harboured a *bla*_{CMY} gene – one alongside *bla*_{CTX-M} group 1 – and one isolate
 94 was *bla*_{DHA-1}-positive. No isolates were positive for *bla*_{SHV} and the remaining
 95 566/1226 (46.2%) isolates were PCR-negative for all GOIs. These isolates were
 96 hypothesised to hyper-produce the chromosomally encoded AmpC β-lactamase;
 97 some of these isolates have been characterised in detail in a separate study (9).

98 Farm- and sample-level risk factors for *bla*_{CTX-M} *E. coli* positivity

99 Based on PCR, carriage of *bla*_{CTX-M} was the most common mechanism of 3GC-
100 resistance in *E. coli* from dairy farms in this study. Identifying management practice-
101 and AMU-associated risk factors for *bla*_{CTX-M} *E. coli* positivity was therefore
102 considered to be an important objective. Overall, 5.4% (224/4145) of samples
103 representing 42/53 (79.2%) of farms contained 3GC-resistant *E. coli* confirmed to
104 carry *bla*_{CTX-M} using PCR. Positivity for *bla*_{CTX-M} *E. coli* was three times higher in Calf
105 samples (samples collected from the environments of calves) (98/631 [15.5%] of
106 samples) than overall (**Table 1**).

107 Given the high positivity rate for *bla*_{CTX-M} *E. coli* in Calf samples, a separate risk
108 factor analysis using only Calf data was performed. One farm-level fixed effect and
109 three sample-level fixed effects were retained in the final multilevel, multivariable
110 logistic regression model (**Table S1, Table 2**). The use of cefquinome or framycetin
111 dry cow therapies were both associated with increased odds of *bla*_{CTX-M} *E. coli*
112 positivity, as was higher average monthly temperature. Plotting sample-level
113 positivity for *E. coli* carrying *bla*_{CTX-M} versus average monthly temperature revealed
114 that the relationship between positivity and temperature was primarily driven by low
115 *bla*_{CTX-M} *E. coli* positivity rates in months where the average temperature was below
116 10°C (**Figure 1A**).

117 Risk factor analysis was also performed for the full dataset. One farm-level fixed
118 effect and three sample-level fixed effects were retained in the final model (**Table**
119 **S2, Table 3**). Interestingly, this model revealed that *bla*_{CTX-M} *E. coli* was less likely to
120 be found in samples obtained from pastureland, which included publicly accessible
121 farmland (Footpaths) compared with other sample types. Analysis of the full dataset
122 confirmed what was seen with the Calf dataset: higher average monthly temperature

was associated with an increased odds of *bla*_{CTX-M} *E. coli* positivity. Again, visualisation of the data confirmed that this was primarily driven by a reduction in *bla*_{CTX-M} *E. coli* positivity rate in months with an average temperature below 10°C (Figure 1B).

A Bayesian logistic regression model was also constructed in which the effect of total farm ABU and specifically total 3GC and 4GC use were tested as predictors for *bla*_{CTX-M} *E. coli* positivity in the total dataset, with 102 potential confounders included. The impact of temperature (odds ratio 1.71 [1.42, 2.05]) on *bla*_{CTX-M} *E. coli* positivity was also retained in this alternative model (Table S3).

Defining sample-level positivity for *bla*_{CTX-M} *E. coli* is dependent upon finding *bla*_{CTX-M} using PCR in *E. coli* colonies that have grown on agar containing cefotaxime. If *bla*_{CTX-M} *E. coli* in a sample exist at such a low density that they are not detected using selective agar, the sample will be falsely identified as negative for *bla*_{CTX-M} *E. coli*. This impact of bacterial density on assay sensitivity is an important consideration in the context of the finding that *bla*_{CTX-M} positivity is low at low temperatures. To account for this, the logistic link function was adjusted (see Supplementary). This only modestly altered the effect sizes and the p-values for the risk factors (Figure S2), confirming that the effect of low temperature on *bla*_{CTX-M} *E. coli* positivity was additional to its effect on *E. coli* prevalence. All values in Tables 2 and 3 come from models with this adjusted logistic link function applied.

Prevalence and risk factor analysis for *E. coli* resistant to other antibacterial classes

All 4145 samples positive for growth of *E. coli* on non-selective agar were also tested for detectable numbers of *E. coli* resistant to four non-cephalosporins: amoxicillin, tetracycline, streptomycin and ciprofloxacin, the last being representative of the HP-CIA class, the fluoroquinolones. Resistance to amoxicillin and tetracycline were the most prevalent types of resistance found, with ciprofloxacin resistance being the least commonly detected (**Table 4**).

Using a Bayesian logistic regression method, factors associated with the risk of a sample being positive for *E. coli* resistant to each of the test ABs were identified from the total dataset. As seen for *bla*_{CTX-M} *E. coli*, where and when the samples were collected were more significantly associated with the odds of finding resistant *E. coli* in a sample than farm-level management practices or ABU, with all four models showing a positive association between average monthly temperature and the odds of finding resistant *E. coli* in a sample (**Table 5**). Also consistent across all models was the significance of sampling different areas of the farm. Again, as with *bla*_{CTX-M} *E. coli*, environments of calves were more likely (as a proportion of all *E. coli* than elsewhere on the farm) to harbour all four types of resistant bacteria. Samples collected from pastureland were, like *bla*_{CTX-M} *E. coli*, negatively associated with the presence of amoxicillin and tetracycline resistance (**Table 5**).

Full results for all variables tested can be found in **Table S4**. Re-running the models with sceptical priors did not affect the results; only very small differences in the model coefficients were observed (**Table S5**). Details of all model checking can be found in Supplementary.

Discussion

Prevalence of *bla*_{CTX-M} positive *E. coli*

This study is unique in its scale: extensive management practice and ABU data along with multiple samples from multiple farms were collected monthly across a two-year period. Overall, 224/4145 (5.4%) of samples were positive for *E. coli* carrying *bla*_{CTX-M}. This compares well with previously calculated *bla*_{CTX-M} *E. coli* carriage of approximately 7% in Danish slaughter pigs (10) and 3.6% in UK broiler chickens and turkeys (11). Various studies have identified much higher prevalence in chicken meat, but this could be due to cross-contamination at slaughter and in the food chain (12, 13).

Studies examining the prevalence of *bla*_{CTX-M} *E. coli* in human populations have shown mixed results. A prevalence of 65.7% was found amongst commensal isolates in Thailand (14). In the UK, a study across four regions reported commensal faecal carriage of *bla*_{CTX-M} *E. coli* to be approximately 7% (15). A recent analysis of human urinary samples from the same region as the farms surveyed in this study gave a sample-level prevalence of *bla*_{CTX-M} *E. coli* of approximately 5% (8). It should be noted that all farm samples in the present study were from faecally contaminated sites, not individual animals, and so it is possible that the number of animals carrying *bla*_{CTX-M} *E. coli* was much lower than the reported sample-level prevalence. Direct comparison with human and other farm animal carriage studies should therefore be made with caution.

Impact of temperature on the odds of finding resistant and *bla*_{CTX-M}-positive *E. coli*

This study found 42/53 (79%) of farms to be positive, based on phenotypic analysis and PCR, for *bla*_{CTX-M} *E. coli*. This was higher than seen in other using similar methodology e.g. 17/48 (35%) of randomly selected UK dairy farms (16) and 5/25 (20%) of farms in Ohio (17) were positive. In the present study, samples were collected each month over two years, hence the chances of finding a positive sample on each farm may have been greater than in these earlier point-prevalence studies (16,17). When farm-level positivity for *bla*_{CTX-M} *E. coli* was plotted on a month-by-month basis (**Figure 2**) the highest prevalence for a single monthly survey was 22.5%, which fits more closely with these other studies.

Sample-level prevalence of *bla*_{CTX-M} *E. coli* was low overall (5.4%). This contrasts with >90% (18) or 50% (19) of *bla*_{CTX-M} *E. coli* in samples taken from bovine faecal pats. This difference could be due to the large number of samples collected here, particularly over winter, given low temperature was associated with low *bla*_{CTX-M} *E. coli* positivity (**Fig. 1A; 1B**). Indeed, the observation that average monthly temperature had a significant effect on *bla*_{CTX-M} *E. coli* positivity (**Table 3**), as well as positivity for resistance to amoxicillin, ciprofloxacin, streptomycin and tetracycline (**Table 5**) highlights problems with studies where a single time-point or sampling season is used. **Figure 2** shows the stark impact of this in real terms: *bla*_{CTX-M} *E. coli* positivity and positivity for ciprofloxacin-resistant *E. coli* at farm level was zero in February, the coldest month of the year (based on average temperature; **Fig 2A, B**). Whilst average annual temperature found at locations across an entire continent has previously been shown to impact average ABR levels at those locations (20), the finding that periods of low temperatures were associated with lower prevalence of a dominant cause of ABR - and particularly HP-CIA resistance at a given location during the course of a year - is particularly important. This observation also leads to

concern about the impact of climate change - and especially increasing temperatures - on attempts to reduce ABR. Whilst temperature was associated with the total number of *E. coli* found in each sample, this was accounted for using a measurement error method incorporated into the model; as such, the effect of temperature on ABR or *bla*_{CTX-M}-positive *E. coli*, whilst in part driven by the effect on total *E. coli* number, also had an independent association suggestive of a temperature-dependent fitness burden of resistance.

High levels of ABR and *bla*_{CTX-M} positive *E. coli* in farm locations dominated by young animals and low levels on pastureland

There were clear differences in the risk of encountering *bla*_{CTX-M} *E. coli* at different sites on a farm (e.g. 15.5% in Calf samples, 4.1% in Adult samples). The Calf environment was also much more likely to have *E. coli* resistant to amoxicillin, tetracycline, streptomycin, and ciprofloxacin, so this seems to be a universal effect.

Other studies have also generally found high levels of resistance in samples collected from or in the environment of younger calves (21-24). There may also be an association with temperature here since calves are generally kept in warmer environments than are adult cows but may also be due to some physiological change with age.

This study also identified a decreased odds of detecting *bla*_{CTX-M} *E. coli* in samples collected on pastureland compared with those collected elsewhere on the farm. This relationship also held for *E. coli* resistant to amoxicillin and tetracycline. Because pastureland may be more affected by the elements, this finding may be partly linked with the association between temperature and ABR.

AB contamination of colostrum as a possible driver of *bla*_{CTX-M} *E. coli* positivity in dairy calves - evidence of direct and co-selection

It has been shown experimentally that feeding waste (AB-contaminated) milk to calves increases faecal excretion of ABR bacteria (25). This practice is reducing on UK dairy farms and, in the analysis presented here, waste milk feeding was not associated with an increased risk of finding ABR or *bla*_{CTX-M} positive *E. coli*. In contrast, the choice of dry cow therapy (an AB preparation inserted into a cow's udder between lactations to help treat or prevent mastitis) was associated with *bla*_{CTX-M} *E. coli* positivity in Calf samples (**Table 2**). It has previously been shown that colostrum from cows given cefquinome dry cow therapy is heavily contaminated with cefquinome (26), and colostrum management is a hugely important part of early life for most farmed mammals and is universally encouraged in dairy farming. In this study, cefquinome (a 4GC) dry cow therapy was most significantly associated with *bla*_{CTX-M} *E. coli* in Calf samples (**Table 2**). This can be explained by direct selection because production of CTX-M confers 4GC resistance in *E. coli* (27). There was also a clear positive association between the usage of framycetin as part of a dry cow therapy combination and the odds of finding *bla*_{CTX-M} *E. coli* in Calf samples (**Table 2**). Whilst no work has been published on the contamination of colostrum with framycetin, its use as a mastitis therapy for milking cows results in identifiable residues in milk (28), so it is highly likely to also contaminate colostrum. It is possible, therefore, that feeding of colostrum - which can be contaminated with AB used for dry cow therapy - is a driver of *bla*_{CTX-M} *E. coli* in calves. An alternative (or indeed an additional) explanation for this observed association is that *E. coli* (a species known to be found in the udders of dairy cows; 29) that carry *bla*_{CTX-M} are selected within the udder during AB dry cow therapy and contaminate colostrum

alongside the AB used. Others (16) also identified overall use of 3/4GCs as a risk factor for *bla*_{CTX-M} *E. coli* presence on dairy farms but have not made a link between the usage of framycetin and prevalence of *bla*_{CTX-M} *E. coli*. However, it is not always clear whether other studies have separated out different dry cow therapies since they have tended to focus on systemic AB use. Clearly, an aminoglycoside like framycetin cannot directly select for *bla*_{CTX-M} *E. coli*, but aminoglycoside resistance genes are common on plasmids (30), as is *bla*_{CTX-M} (27) and so this may be an example of co-selection, where selection of resistance to one antibacterial class increases resistance to another.

Conclusions

Overall, we provide important evidence that sampling at single time-points and/or limited locations on a farm are unlikely to be adequate to accurately determine the status of the farm concerning the prevalence of ABR *E. coli*. This makes comparisons between surveillance studies on farms, designed for research or for regulatory purposes, extremely difficult, and we urge for a standardised, multi-sample framework accounting for the differential risk factors identified here to be used in the design of future studies.

Materials and Methods

Farm recruitment and ethical approval

A convenience sample of 53 dairy farms was recruited through personal contacts, local veterinary practices, and milk processors. Details of the study population are presented in Supplementary. Of these, 43 farms were in a 50 x 50 km area defined based on the locations of 146 general practices that referred routine urine samples from human patients to the microbiology reference lab at Severn Pathology, Southmead Hospital (8). A further 10 study farms were clustered in a separate region in South West England. All farmers gave fully informed consent to participate in the study. Ethical approval was obtained from the University of Bristol's Faculty of Health Sciences Research Ethics Committee (ref 41562).

Farm sampling and sample processing

Farms were visited monthly between January 2017 and December 2018. Samples were collected using sterile overshoes (over-boot socks) traversing farm areas. Where access was restricted (e.g. for pens containing single or pairs of calves), samples were collected directly from the ground using gloved hands. Details of the six types of samples collected are in Supplementary; these represent faecally contaminated environments representative of milking cows (Adult), cows between periods of lactation (Dry Cow), heifers after weaning (Heifer), heifers before weaning (Calf). Samples of these types collected from pastureland were designated as such for separate analysis (Pasture) which also included samples collected from publicly accessible land on the farm (Footpath). Samples were refrigerated from collection to processing, were transferred into individual labelled sterile stomacher bags, and

suspended in 10 ml.g⁻¹ of phosphate buffered saline (PBS Dulbecco A; Oxoid, Basingstoke, UK). Samples were then mixed for one min in a stomacher (Stomacher 400, Seward, Worthing, UK). Samples were mixed 50:50 with sterile glycerol and aliquots stored at -80°C.

Microbiology and PCR analysis

Twenty microlitres of sample (diluted 1:10) were spread onto tryptone bile X-glucuronide agar (TBX; Scientific Laboratory Supplies); 20 µL of undiluted sample were spread onto TBX agar containing 16 mg.L⁻¹ tetracycline, 8 mg.L⁻¹ amoxicillin, 0.5 mg.L⁻¹ ciprofloxacin, 16 mg.L⁻¹ streptomycin or 16 mg.L⁻¹ cephalixin. Plates were incubated at 37°C, and the number of blue colonies (*E. coli*) counted. Samples yielding no *E. coli* colonies on antibiotic-free agar were excluded from further analysis. Up to five *E. coli* isolates from each cephalixin TBX agar plate were transferred onto cefotaxime (CTX, 2 mg.L⁻¹) TBX agar. All AB concentrations were chosen as those which define clinically relevant resistance in humans according to EUCAST (31). Two multiplex PCRs were performed to screen for β-lactamase genes in CTX-R *E. coli*. The first was to detect *bla*_{CTX-M} groups as previously described (32) and the second was to detect the following additional β-lactamase genes: *bla*_{CMY}, *bla*_{DHA}, *bla*_{SHV}, *bla*_{TEM}, *bla*_{OXA-1} (8).

Risk factor analyses

The risk factors examined fell into four categories: farm management, ABU, sample characteristics and meteorological. Four management practice questionnaires were developed (details provided in Supplementary). ABU was extracted from prescribing

and sales data between Jan 2016 to Dec 2018 obtained from veterinary practices servicing the study farms. For two farms, sales data were not available and on-farm records were used. The full method for obtaining, cleaning, and processing ABU data can be found in Supplementary. Local meteorological data were extracted from publicly available UK Met Office data (<https://www.metoffice.gov.uk/pub/data/weather/uk/climate/stationdata/yeoviltontdata.txt>).

Sample processing and data analysis workflows are illustrated in **Figure S1**. All data analysis was performed using R (<https://www.r-project.org/>). Two modelling approaches were used: 1) variable selection via univariable screening and stepwise model selection with a multilevel, multivariable logistic regression model and 2) a regularised Bayesian model. Both were used to analyse risk factors associated with *bla*_{CTX-M} *E. coli* positivity; the second was also used to analyse risk factors associated with positivity for *E. coli* resistant to amoxicillin, ciprofloxacin, streptomycin, and tetracycline. Sensitivity analyses were performed to test for measurement bias, to account for the fact that resistant *E. coli* were more likely to be found in a sample if there was a higher density of bacterial colony forming units. Further details of variable selection and development of the models and model checking are provided in Supplementary.

Acknowledgements

We wish to thank all the farmers and veterinary surgeons who participated in this study. In addition, we would like to thank the milk processing companies who supported our recruitment process and the veterinary students who helped with data

processing and sample collection. Genome sequencing was provided by
MicrobesNG (<http://www.microbesng.uk>), which is supported by the BBSRC (grant
number BB/L024209/1).

Funding

This work was funded by grant NE/N01961X/1 to M.B.A., K.K.R., K.M.T., T.A.C. and
D.C.B. from the Antimicrobial Resistance Cross Council Initiative supported by the
seven United Kingdom research councils. R.A. was supported by the Jean Golding
Institute for data-intensive research at the University of Bristol. G.M.R. was funded
by the Langford Trust.

Transparency declarations

D.C.B. was president of the British Cattle Veterinary Association 2018-19.
Otherwise, the authors declare no competing interests. Farming and veterinary
businesses who contributed data and permitted access for sample collection were
not involved in the design of this study or in data analysis and were not involved in
drafting the manuscript for publication.

Author Contributions

Conceived the Study: D.C.B., K.K.R., M.B.A.

Collection of Data: H.S., J.F., K.M., E.F.P., O.M., V.C.G., supervised by T.A.C.,
K.K.R., G.M.R., M.B.A.

379 Cleaning and Analysis of Data: H.S., J.F., R.A., V.C.G., E.F.P, L.V., M.E., supervised
380 by K.M.T., K.K.R., M.B.A.

381 Initial Drafting of Manuscript: H.S., K.K.R., M.B.A.

382 Corrected and Approved Manuscript: All Authors

383 **Tables**

384 **Table 1.** Prevalence of *E. coli* carrying *bla*_{CTX-M} at farm and sample levels

Sample type		Farm level	Sample level
Overall	Total sample size	53	4145
	Total (%) positive for CTX-M-carrying <i>E. coli</i>	42 (79.2%)	224 (5.4%)
Adult	Total sample size	52	1835
	Total (%) positive for CTX-M-carrying <i>E. coli</i>	25 (48.1%)	76 (4.1%)
Dry Cow	Total sample size	46	282
	Total (%) positive for CTX-M-carrying <i>E. coli</i>	7 (15.2%)	8 (2.8%)
Calf	Total sample size	51	631
	Total (%) positive for CTX-M-carrying <i>E. coli</i>	33 (64.7%)	98 (15.5%)
Heifer	Total sample size	41	1235
	Total (%) positive for CTX-M-carrying <i>E. coli</i>	18 (44%)	40 (3.2%)
Pastureland	Total sample size	47	630
	Total (%) positive for CTX-M-carrying <i>E. coli</i>	8 (17%)	12 (1.9%)
Pastureland that is publicly accessible (Footpath)	Total sample size	41	395
	Total (%) positive for CTX-M-carrying <i>E. coli</i>	8 (20.0%)	11 (2.8%)

385

386

Table 2. Fixed effects from the multilevel, multivariable logistic regression model predicting *bla*_{CTX-M} *E. coli* positivity performed on Calf samples

Risk factor	Odds ratio [95% confidence interval]	p
Use of cefquinome dry cow therapy in the last six months	4.18 [2.11, 8.25]	0.00003
Daily water trough cleaning	0.44 [0.29, 0.69]	0.0002
Average monthly temperature	1.57 [1.20, 2.06]	0.0008
Use of framycetin dry cow therapy in the last six months	1.91 [1.01, 3.61]	0.04

390 **Table 3.** Fixed effects from the multilevel, multivariable logistic regression model
 391 predicting *bla*_{CTX-M} *E. coli* positivity performed on the full dataset

Risk factor	Odds ratio [95% confidence interval]	p
Sample taken from the environment of pre-weaned heifers	4.52 [3.25, 6.27]	<0.00000001
Average monthly temperature	1.61 [1.36, 1.90]	0.00000001
Sample taken from pastureland	0.32 [0.17,0.61]	0.0004
Feeding of maize silage	3.28 [1.50, 7.18]	0.002

392

393 **Table 4.** Farm and sample-level prevalence of resistance to non-cephalosporins

Antibacterial drug	Farm level resistance	Sample level resistance
Amoxicillin	53/53 (100%)	2754/4145 (66%)
Ciprofloxacin	49/53 (92%)	263/4145 (6%)
Streptomycin	53/53 (100%)	1475/4145 (36%)
Tetracycline	53/53 (100%)	2874/4145 (69%)

394

Table 5. Fixed effects from a Bayesian model predicting resistant *E. coli* positivity performed on the full dataset

Risk factor	Odds ratio [95% credible interval]
Amoxicillin resistance	
Average monthly temperature	1.91 [1.57, 2.35]
Sample taken from the environment of pre-weaned heifers	1.99 [1.29, 2.98]
Sample taken from pastureland	0.27 [0.20, 0.37]
Ciprofloxacin resistance	
Average monthly temperature	2.14 [1.63, 2.87]
Sample taken from the environment of pre-weaned heifers	4.13 [2.79, 6.46]
Streptomycin resistance	
Average monthly temperature	1.53 [1.32, 1.77]
Sample taken from the environment of pre-weaned heifers	1.95 [1.46, 2.51]
Tetracycline resistance	
Average monthly temperature	1.98 [1.55, 2.55]
Sample taken from the environment of pre-weaned heifers	3.39 [2.00, 5.82]
Sample taken from pastureland	0.24 [0.15, 0.35]
Sample taken during the calving season	2.02 [1.10, 3.29]
Average monthly rainfall	1.26 [1.08, 1.47]
Total farm streptomycin use in 2017 measured in mg/PCU*	0.76 [0.60, 0.97]
Total farm ABU in 2017 measured in mg/PCU*	1.47 [1.01, 2.13]

References

1. <https://www.gov.uk/government/collections/antimicrobial-resistance-amr-information-and-resources>. 2019.
2. ESVAC. European Surveillance of Veterinary Antimicrobial Consumption.
3. Veterinary Medicines Directorate & Animal and Plant Health Agency. 2015. UK Veterinary Antibiotic Resistance and Sales Surveillance.
4. Veterinary Medicines Directorate & Animal and Plant Health Agency. 2018. UK Veterinary Antibiotic Resistance and Sales Surveillance.
5. Lazarus B, Paterson DL, Mollinger JL, Rogers BA. 2014. Do Human Extraintestinal *Escherichia coli* Infections Resistant to Expanded-Spectrum Cephalosporins Originate From Food-Producing Animals? A Systematic Review. Clin Infect Dis 60:439-452.
6. Bevan ER, Jones AM, Hawkey PM. 2017. Global epidemiology of CTX-M β -lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145-2155.
7. Madec J-Y, Haenni M. 2018. Antimicrobial resistance plasmid reservoir in food and food-producing animals. Plasmid 99:72-81.
8. Findlay J, Gould VC, North P, Bowker KE, Williams OM, MacGowan AP, Avison MB. 2020. Characterisation of cefotaxime-resistant urinary *Escherichia coli* from primary care in South-West England 2017-2018. J Antimicrob Chemother 75:65-71.
9. Alzayn M, Findlay J, Schubert H, Mounsey O, Gould VC, Heesom KJ, Turner KM, Barrett DC, Reyher KK, Avison MB. 2020. Characterisation of AmpC Hyper-Producing *Escherichia coli* from Humans and Dairy Farms Collected in Parallel in the Same Geographical Region. bioRxiv doi:10.1101/784694; J Antimicrob Chemother (In Press) doi:10.1093/jac/dkaa207.
10. Agersø Y, Aarestrup FM, Pedersen K, Seyfarth AM, Struve T, Hasman H. 2011. Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. J Antimicrob Chemother 67:582-588.

- 430 11. Randall LP, Clouting C, Horton RA, Coldham NG, Wu G, Clifton-Hadley FA,
431 Davies RH, Teale CJ. 2010. Prevalence of *Escherichia coli* carrying extended-
432 spectrum β -lactamases (CTX-M and TEM-52) from broiler chickens and
433 turkeys in Great Britain between 2006 and 2009. J Antimicrob Chemother
434 66:86-95.
- 435 12. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck
436 M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X,
437 Kluytmans J. 2011. Extended-spectrum β -lactamase genes of *Escherichia coli*
438 in chicken meat and humans, The Netherlands. Emerg Infect Dis 17:1216-
439 1222.
- 440 13. Kola A, Kohler C, Pfeifer Y, Schwab F, Kühn K, Schulz K, Balau V, Breitbach
441 K, Bast A, Witte W, Gastmeier P, Steinmetz I. 2012. High prevalence of
442 extended-spectrum- β -lactamase-producing Enterobacteriaceae in organic and
443 conventional retail chicken meat, Germany. J Antimicrob Chemother 67:2631-
444 2634.
- 445 14. Luvsansharav U-O, Hirai I, Nakata A, Imura K, Yamauchi K, Niki M,
446 Komalamisra C, Kusolsuk T, Yamamoto Y. 2012. Prevalence of and risk
447 factors associated with faecal carriage of CTX-M β -lactamase-producing
448 Enterobacteriaceae in rural Thai communities. J Antimicrob Chemother
449 67:1769-1774.
- 450 15. McNulty CAM, Lecky DM, Xu-McCrae L, Nakiboneka-Ssenabulya D, Chung
451 K-T, Nichols T, Thomas HL, Thomas M, Alvarez-Buylla A, Turner K, Shabir S,
452 Manzoor S, Smith S, Crocker L, Hawkey PM. 2018. CTX-M ESBL-producing
453 Enterobacteriaceae: estimated prevalence in adults in England in 2014. J
454 Antimicrob Chemother 73:1368-1388.
- 455 16. Snow LC, Warner RG, Cheney T, Wearing H, Stokes M, Harris K, Teale CJ,
456 Coldham NG. 2012. Risk factors associated with extended spectrum beta-
457 lactamase *Escherichia coli* (CTX-M) on dairy farms in North West England
458 and North Wales. Prev Vet Med 106:225-234.
- 459 17. Mollenkopf DF, Weeman MF, Daniels JB, Abley MJ, Mathews JL, Gebreyes
460 WA, Wittum TE. 2012. Variable within- and between-herd diversity of CTX-M
461 cephalosporinase-bearing *Escherichia coli* isolates from dairy cattle. Appl
462 Environ Microbiol 78:4552-4560.

- 463 18. Horton RA, Duncan D, Randall LP, Chappell S, Brunton LA, Warner R,
464 Coldham NG, Teale CJ. 2016. Longitudinal study of CTX-M ESBL-producing
465 *E. coli* strains on a UK dairy farm. Res Vet Sci 109:107-113.
- 466 19. Brunton LA, Reeves HE, Snow LC, Jones JR. 2014. A longitudinal field trial
467 assessing the impact of feeding waste milk containing antibiotic residues on
468 the prevalence of ESBL-producing *Escherichia coli* in calves. Prev Vet Med
469 117:403-412.
- 470 20. MacFadden DR, McGough SF, Fisman D, Santillana M, Brownstein JS. 2018.
471 Antibiotic Resistance Increases with Local Temperature. Nat Clim Chang
472 8:510-514.
- 473 21. Watson E, Jeckel S, Snow L, Stubbs R, Teale C, Wearing H, Horton R,
474 Toszeghy M, Tearne O, Ellis-Iversen J, Coldham N. 2012. Epidemiology of
475 extended spectrum beta-lactamase *E. coli* (CTX-M-15) on a commercial dairy
476 farm. Vet Microbiol 154:339-346.
- 477 22. Duse A, Persson Waller K, Emanuelson U, Ericsson Unnerstad H, Persson Y,
478 Bengtsson B. 2016. Occurrence and Spread of Quinolone-Resistant
479 *Escherichia coli* on Dairy Farms. Appl Environ Microbiol 82:3765-3773.
- 480 23. Pereira RV, Siler JD, Ng JC, Davis MA, Grohn YT, Warnick LD. 2014. Effect
481 of on-farm use of antimicrobial drugs on resistance in fecal *Escherichia coli* of
482 preweaned dairy calves. J Dairy Sci 97:7644-7654.
- 483 24. Afema JA, Davis MA, Sisco WM. 2019. Antimicrobial use policy change in
484 pre-weaned dairy calves and its impact on antimicrobial resistance in
485 commensal *Escherichia coli*: a cross sectional and ecological study. BMC
486 Microbiol 19:217-217.
- 487 25. Awosile BB, Smith BA. 2017. Risk assessment modelling of fecal shedding
488 caused by extended-spectrum cephalosporin-resistant *Escherichia coli*
489 transmitted through waste milk fed to dairy pre-weaned calves. J Dairy Sci
490 100:9667-9673.
- 491 26. Bachmann J, Helmschrodt C, Richter A, Heuwieser W, Bertulat S. 2018.
492 Residue concentration of cefquinome after intramammary dry cow therapy
493 and short dry periods. J Dairy Sci 101:7540-7550.
- 494 27. Peirano G, Pitout JDD. 2019. Extended-Spectrum β -Lactamase-Producing
495 Enterobacteriaceae: Update on Molecular Epidemiology and Treatment
496 Options. Drugs. 79:1529-1541.

- 497 28. Luo PJ, Zhang JB, Wang HL, Chen X, Wu N, Zhao YF, Wang XM, Zhang H,
498 Zhang JY, Zhu L, Jiang WX. 2016. Rapid and Sensitive Chemiluminescent
499 Enzyme Immunoassay for the Determination of Neomycin Residues in Milk.
500 Biomed Environ Sci 29:374-378.
- 501 29. Bradley AJ, Green MJ. 2001. Adaptation of *Escherichia coli* to the bovine
502 mammary gland. J Clin Micro 39:1845-1849.
- 503 30. Schultsz C, Geerlings S. 2012. Plasmid-mediated resistance in
504 Enterobacteriaceae: changing landscape and implications for therapy. Drugs.
505 72:1-16.
- 506 31. European Committee on Antimicrobial Susceptibility Testing. 2020.
507 [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_ta](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf)
508 [bles/v_9.0_Breakpoint_Tables.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf). Last accessed May 2020.
- 509 32. Woodford N, Fagan EJ, Ellington MJ. 2005. Multiplex PCR for rapid detection
510 of genes encoding CTX-M extended-spectrum β -lactamases. J Antimicrob
511 Chemother 57:154-155.

Figure Legends

Figure 1. Average monthly temperature vs. presence (1) or absence (0) of *E. coli* positive for *bla*_{CTX-M} in samples from (A) pre-weaned calves and (B) all faecally contaminated dairy farm environments. Each sample is represented by a dot. A multilevel, multivariable logistic regression model revealed a positive association with increased temperature in both cases ($p < 0.0001$).

Figure 2. Percentage of farms positive for (A) *E. coli* positive for *bla*_{CTX-M} in samples (B) ciprofloxacin resistant *E. coli*. Data are presented by month (bars) and overlayed by a graph of average monthly temperature (dots) representing a year during the middle period of this study. Samples from calves have been excluded.

523 **Figure 1**

524 **A**

526 **B**

528 **Figure 2**

529 **A**

531 **B**