

# The effects of calves fed colostrum from dairy cows treated with first generation cephalosporin dry cow treatments on selection and spread of antimicrobial resistance in dairy calves

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## INTRODUCTION

Transmission of Extended Spectrum Beta Lactamase producing Enterobacteriaceae (ESBL-pE) from animals to humans is considered a huge risk for human health. A possible route could be transmission of ESBL-pE from the dam via faeces or colostrum. First generation cephalosporins (1GC) are globally used in dairy cows for dry cow therapy (DCT) but may have the potential to select for ESBL producing bacteria while this potential is perceived to be lower for narrow spectrum penicillin (NSP).

## OBJECTIVE

In this study, we compared the effects of a 1GC versus a NSP DCT on selection and spread of cefotaxime resistant (CTX-R) *E. coli* in the gut of the neonatal calf.

## MATERIALS AND METHODS

- ▶ On three commercial Dutch dairy farms known to harbour ESBL *E. coli* in their herd, cows before drying off were screened for CTX-R resistant *E. coli* in their faeces using selective enrichment of 1mg/ml CTX in LB broth followed by plating on MacConkey agar. ESBL *E. coli* positive cows were randomized to receive standard NSP DCT (600mg cloxacillin) or 1GC DCT (300mg cefapirin) at drying off. Post-calving, calves were fed their dams' colostrum for 2-3 days. Faecal samples of the neonatal calves born from these cows were taken at day 2, day 7 and day 14 after birth. Samples were stored under cooled conditions (2-8 °C) and sent to the laboratory within 48 hours. Faeces was enriched in LB broth with 1mg/ml CTX, incubated overnight and plated on MacConkey agar for screening of the presence of CTX resistant *E. coli*. Confirmation of *E. coli* occurred with MALDI-TOF. If positive, quantitative analyses was performed to calculate CTX-R *E. coli* CFU/gram faeces. Fresh faeces was mixed with saline and 10-fold dilutions were plated on MacConkey + CTX and incubated overnight for quantitative counts.
- ▶ A one-way ANCOVA test was used to assess whether a difference in counts between the two groups could be observed with time points of sampling as covariate and farms as random factor.
- ▶ DNA of CTX-R isolates was isolated and sequenced using Nanopore MinION and their resistance genes and mutations were determined using AMRFinderPlus. Phylogenetic trees were constructed using Mashtree and visualized using iTOL.

Drying off cows, harbouring CTX-R *E. coli* in their gastrointestinal tract, with either cloxacillin or cefapirin did not result in significantly different faecal CTX-R *E. coli* counts in neonatal calves born from these cows.

Variations in faecal CTX-R *E. coli* counts were large in calves within both study groups, indicating that possibly, other cow or farm related factors are of greater influence for selection and transmission of ESBL-pE via the calf.



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## RESULTS

- ▶ A total of 172 cows were screened for the presence of CTX-R *E. coli* in their faeces before drying off. 22 cows (13%) tested positive on CTX-R *E. coli* and were included in the study of which half received 1GC and others NSP DCT.
- ▶ Results from the samples taken from calves (Figure 2). On day 2, all calves harboured CTX-R *E. coli*; on day 7 CTX-R *E. coli* could not be found in one calf in the 1GC group and in three calves of the NSP group; on day 14 CTX-R *E. coli* could not be found in one calf in the 1GC group.

FIGURE 1. Schematic overview of activities during the trial.

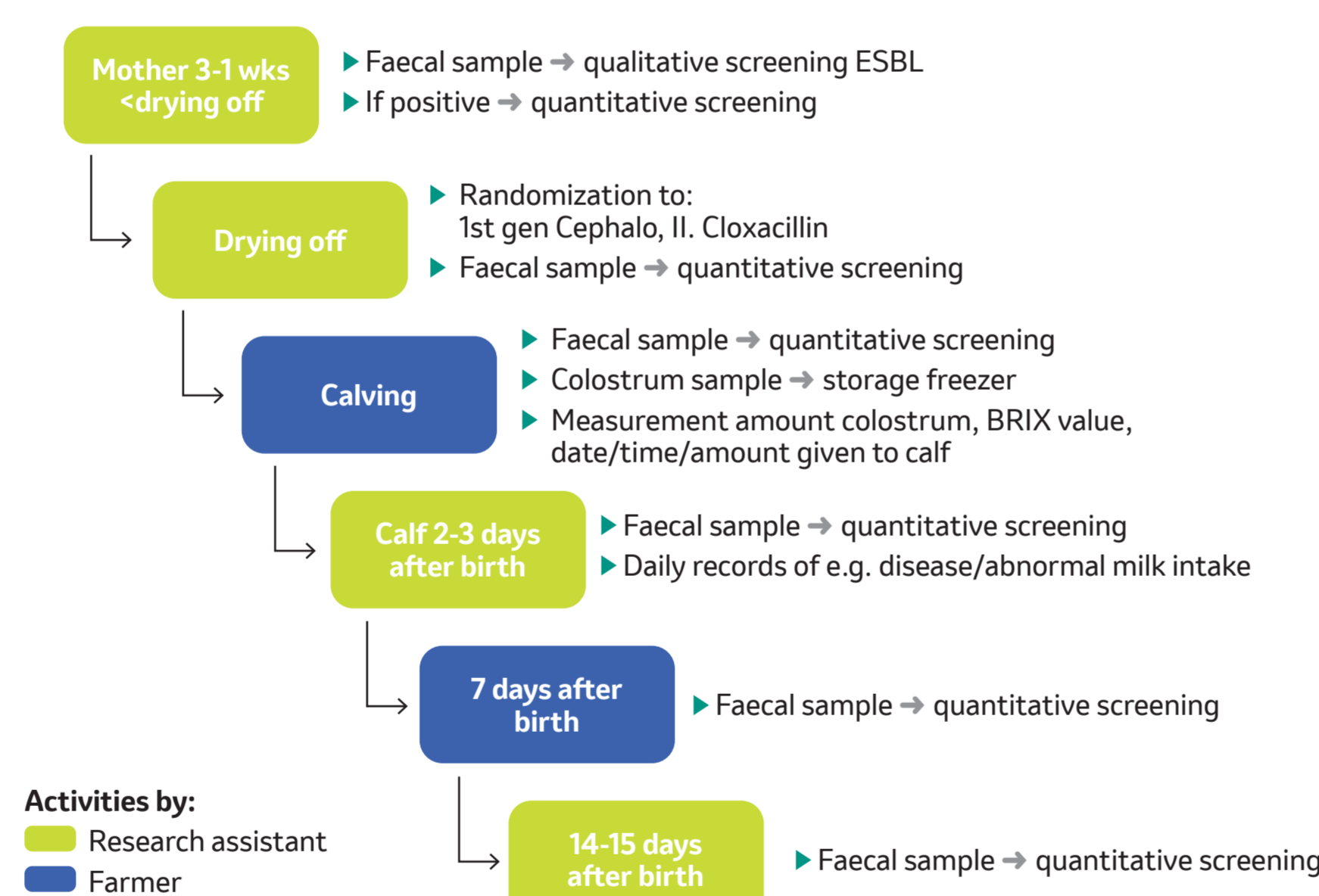
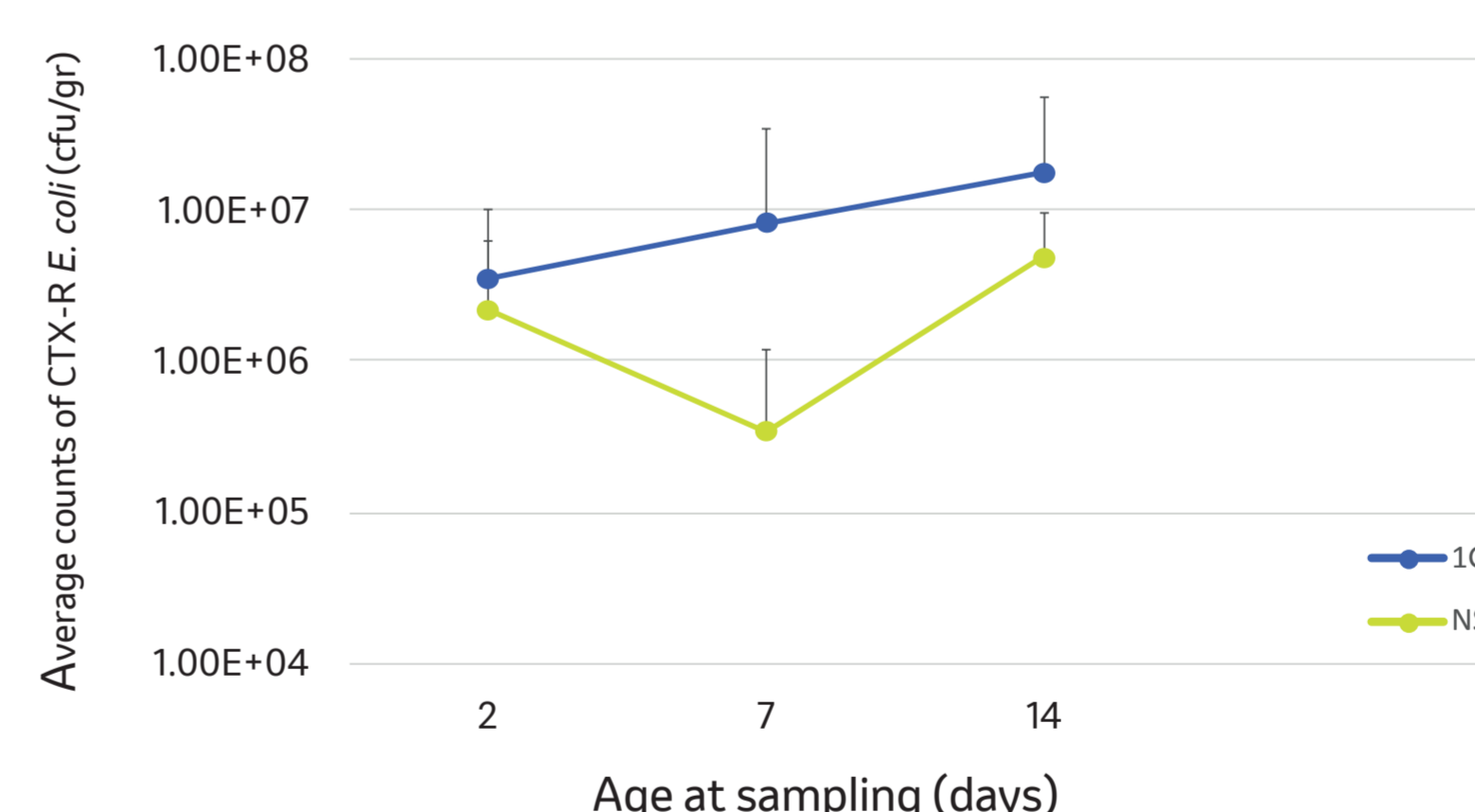


FIGURE 2. Average CTX-R *E. coli* counts and 95% CI in feces of a newborn calf at 3 time points after birth, 1: day 2, 2: day 7, and 3: day 14, that received colostrum of an ESBL positive mother treated in all 4 quarters with Faecal (1GC, cefapirin 300 mg) or Orbenin DC Extra (NSP, cloxacillin 600 mg) at drying off (n=10-12 calves in each treatment group).



## RESULTS

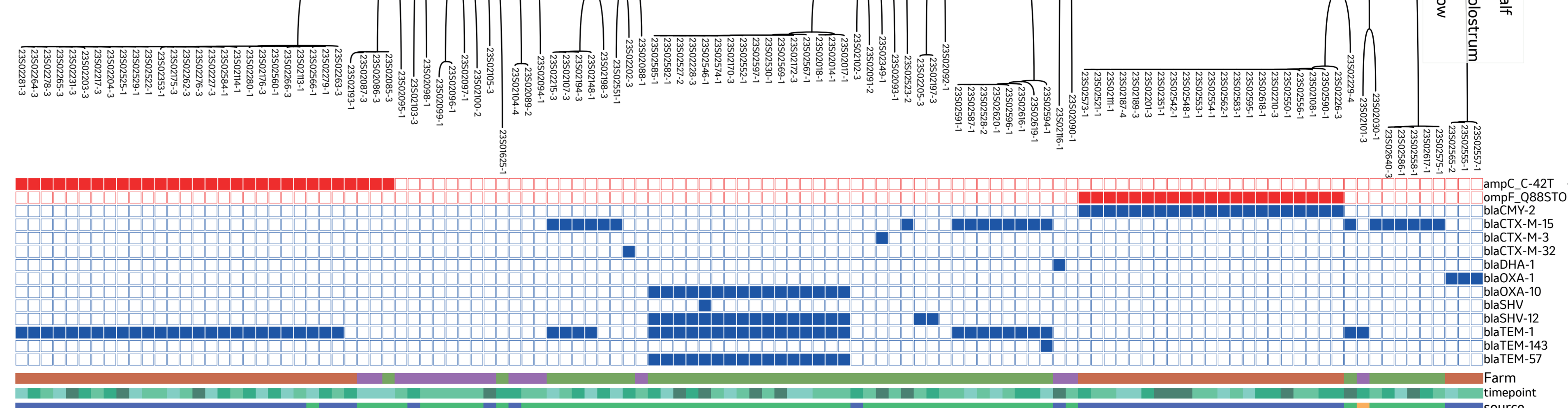
Although on each sampling moment the quantitative counts of CTX-R *E. coli* were on average higher in calves born from dams that had received 1GC, no significant differences in CFU counts per gram faeces could be observed compared to calves born from dams receiving cloxacillin DCT in the total study period (F=2.401; p=0.20) when adjusted for farm and sampling time point. Further analysis based on Whole Genome Sequencing of the isolated CTX-R *E. coli* is pending.

## RESULTS

Results of genome sequencing of DNA of CTX-R isolates is shown in Figure 3. We found ESBL resistance (CTX-M, SHV12 (blue boxes), cefotaxim resistance (AmpC promoter mutation (red boxes), CMY-2 (blue boxes), and an OmpF truncating mutation (red) together with CMY-2. This mutation is associated with decreased susceptibility for all betalactams (also 3rd gen cephalosporins and carbapenems).

FIGURE 3. Results of genomes sequencing of DNA of CTX-R isolates, showing resistance genes, mutations and a phylogenetic tree. Sources of isolates are from 3 different dairy farms and from the mother cow, her colostrum and her calf.

Timepoint 1: 2 weeks before  
Timepoint 2: at drying off  
Timepoint 3: 1 week after drying off  
Timepoint 4: at calving.



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