



# Dynamics of faecal carriage of ESBL-producing *Escherichia coli* in dairy cattle

Egil A.J. Fischer<sup>1,\*</sup> | Joost Hordijk<sup>1</sup> | Tine van Werven<sup>1,2</sup> | Steven Sietsma<sup>2</sup> | Liese Van Gompel<sup>4</sup> | Dick Heederik<sup>4</sup> | Jaap Wagenaar<sup>1,3</sup> | Mirjam Nielen<sup>1</sup> | Arjan Stegeman<sup>1</sup>

<sup>1</sup> Utrecht University, Faculty of Veterinary Medicine  
\*e.a.j.fischer@uu.nl

<sup>2</sup> Universitaire Landbouwhuisdieren Praktijk

<sup>3</sup> Central Veterinary Institute, part of Wageningen UR

<sup>4</sup> Utrecht University, Institute for Risk Assessment Sciences

**Aims and objectives:** Although the presence of extended spectrum beta-lactamases producing *E. coli* (ESBL) in dairy cattle has been reported, little is known about its dynamics within herds. Such knowledge is important to identify control options. The purpose of this study was **to determine the dynamics of faecal carriage of ESBLs in dairy cattle**

Farm	Samples per age-group				ESBL pos.	Antimicr. treatments*	Calves	
	1	2	3	4			Total	Treatment**
4	13	21	33	1	5	6	32	16
14	31	48	39	32	13	4	50	32
15	0	35	45	8	18	7	19	3
19	7	35	30	3	26	2	35	8
<b>Total</b>	<b>51</b>	<b>139</b>	<b>147</b>	<b>44</b>	<b>62</b>	<b>19</b>	<b>136</b>	<b>59</b>

Table 1: Description of the data included in the analyses. Animals with previous positive ESBL sample were excluded in further analyses. \* Number of treatments in period prior to sampling \*\*Treatment of mother with penicillin dry-off therapy.

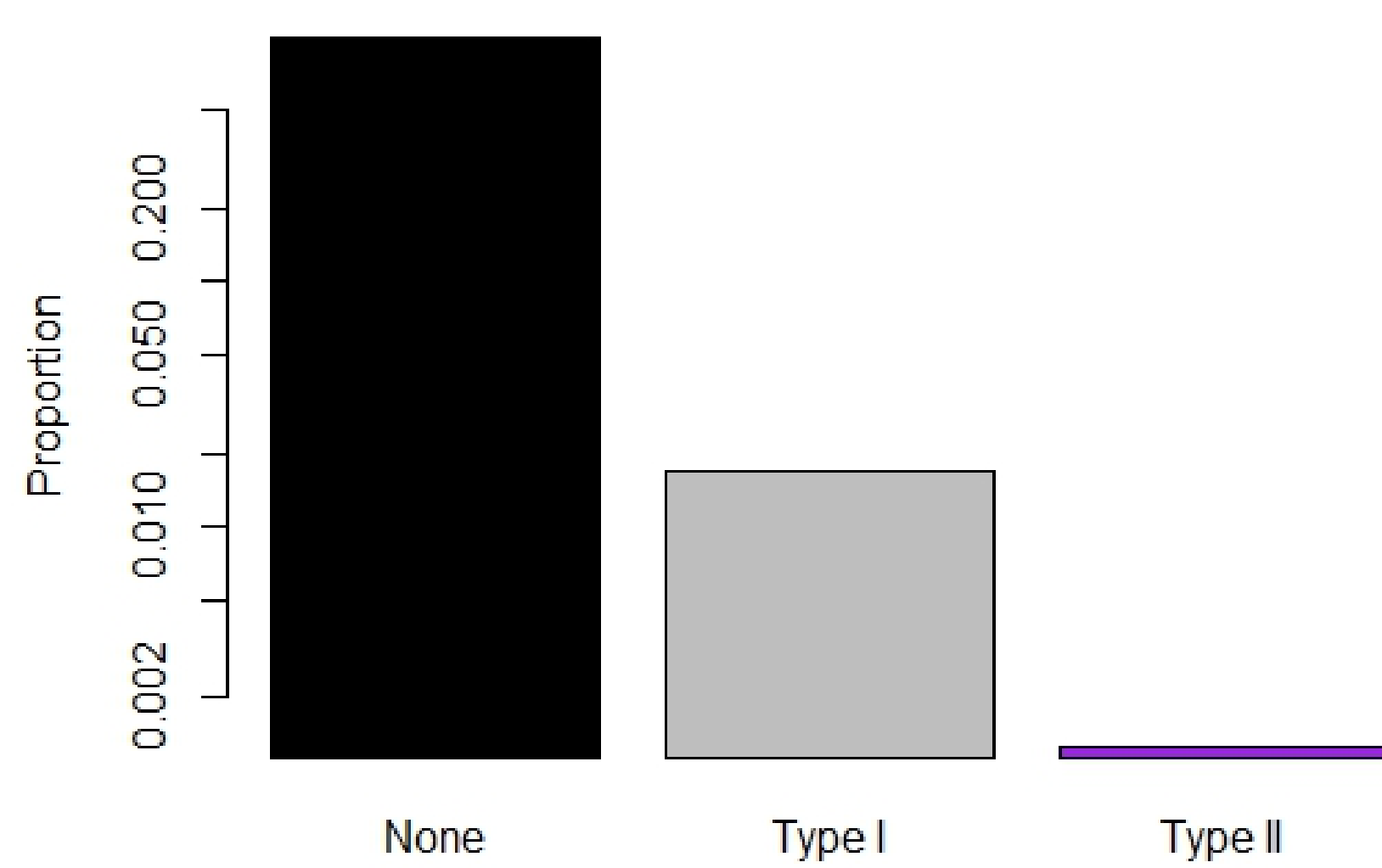


Figure 2 Proportion of time at risk after usage of antibiotics in dairy cattle on selected farms, classification of Netherlands Veterinary Medicines Authority (SDa). Type II have a high selection risk for ESBLs

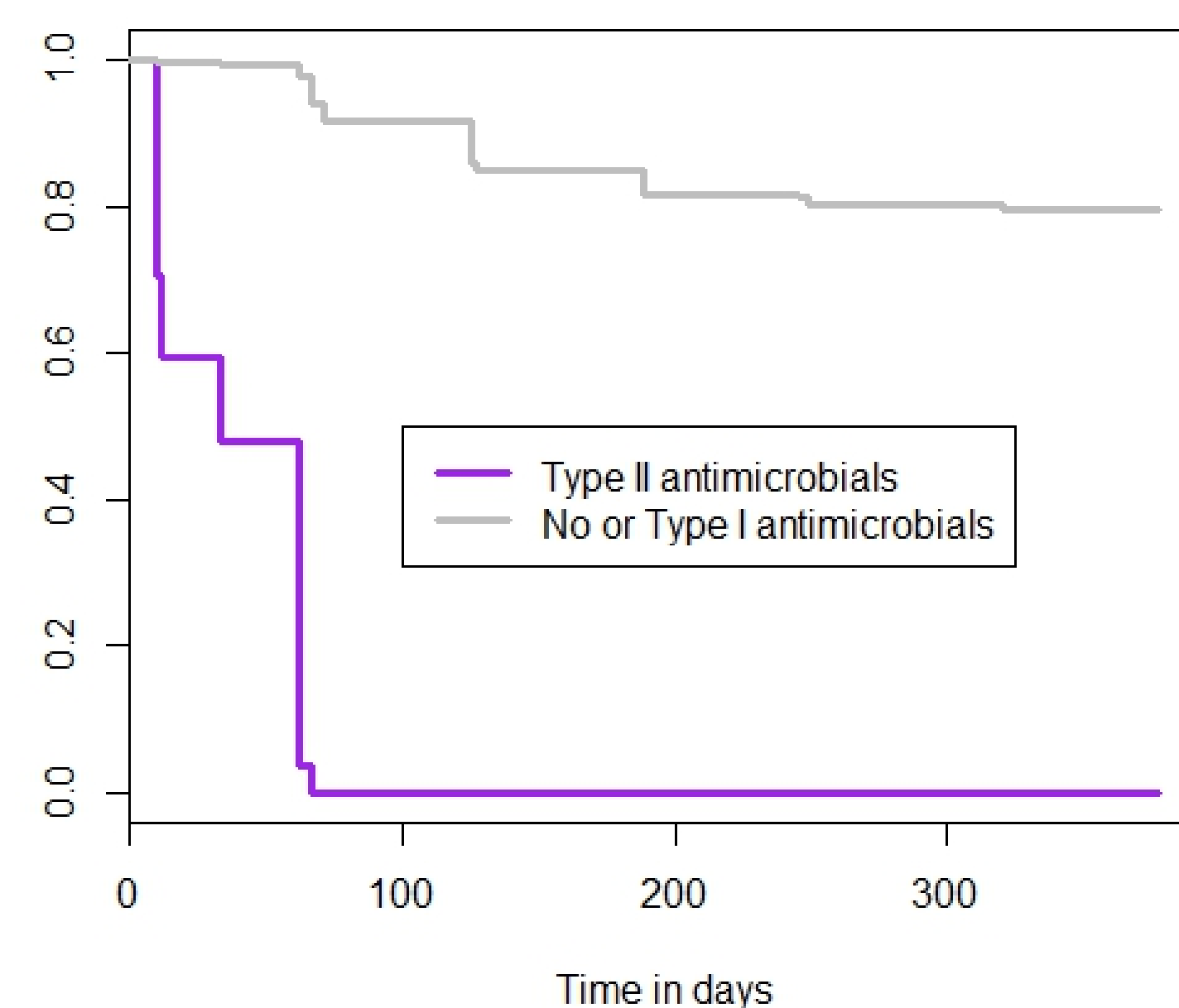


Figure 3 Survival-plot of time until positive faecal sample following antimicrobial treatment with Type II or not.

**Type II antibiotics**  
log hazard = 4.9 PAF = 0.16

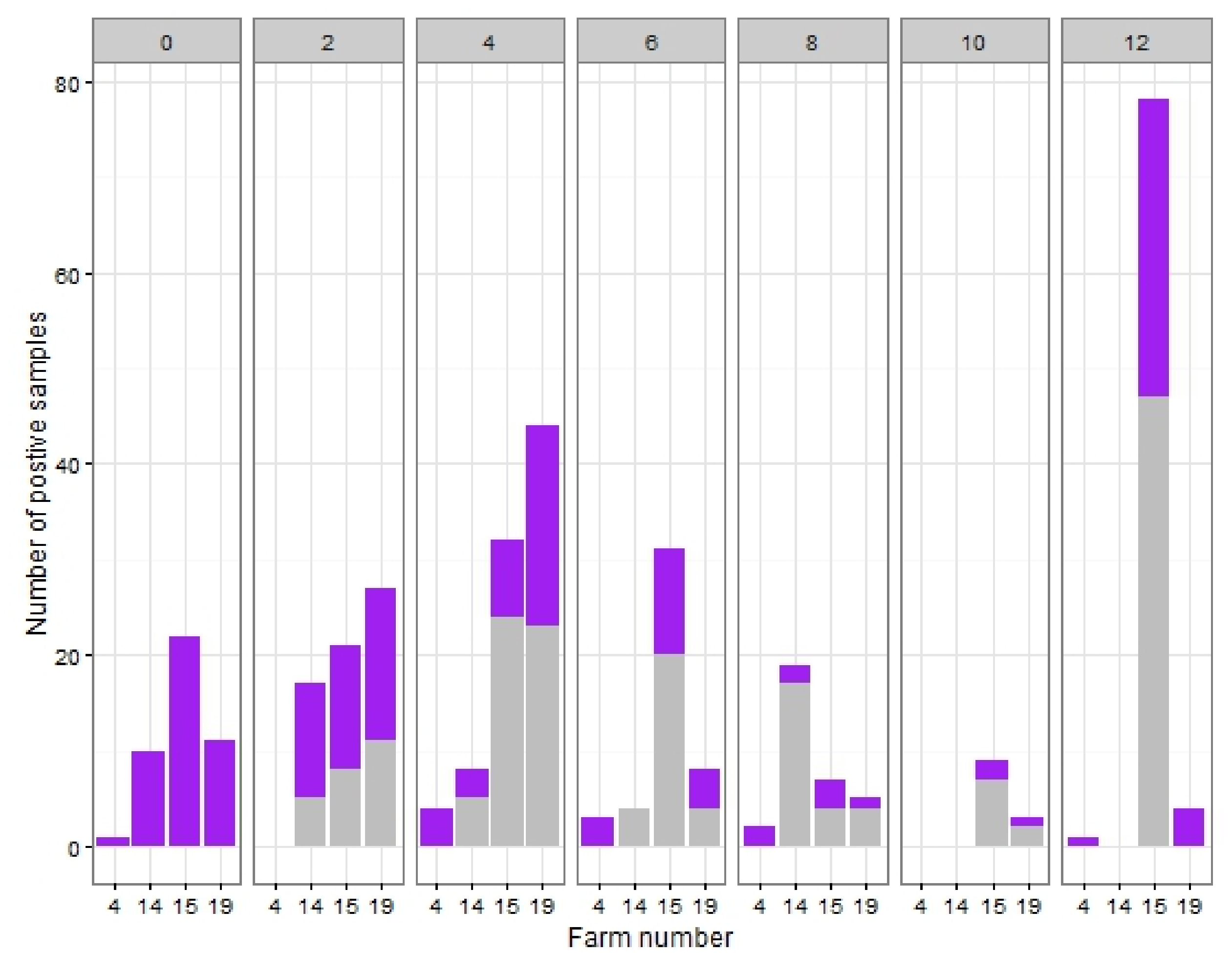


Figure 1 Number of ESBL positive faecal samples of cows. Purple indicates the number of cows that were not previously found positive. Grey indicate previously positive animals.

		Penicillin dry-off therapy		
		Yes	No	Total
Calf sample	Positive	11	19	30
	Negative	48	58	106
	Total	59	77	136

Table 2: Cross-table of ESBL positive first sample in a calf and antimicrobial usage in the mother for drying off prior to feeding colostrum (p = 0.53). Penicillin was used in the dry-off therapy.

**Material and methods:** Initially (T0), 20 Dutch farms with a relatively high antimicrobial usage were selected and approximately 100 samples were taken from individual animals on each farm distributed over 4 age groups. On 10 farms with the highest ESBL prevalence sampling was continued every 2 months for 1 year. At each sampling, all new animals in the youngest age group, all animals that had moved to another group were sampled by rectal sampling. Individual samples were cultured in Luria-Bertani broth supplemented with 1 mg/L cefotaxime and subsequently streaked onto MacConkey agar supplemented with 1 mg/L cefotaxime (MCC). In parallel, a dilution range was made of all samples, which were subsequently inoculated on MCC for quantitative analysis. ESBL suspected isolates were screened by PCR and sequencing analysis.

**Statistical analysis:** Eight out of ten farms were totally negative or only had a few positive samples in the first sampling round, for the analyses we selected 4 farms on which ESBL positive samples were found (farm 15 & 19 with most positive samples, and farm 4 & 14 with a few positive samples at T0) were taken into account. We applied Cox-proportional hazard model to the time until first positive sample. Risk factor was antibiotics no/Type I antimicrobials versus Type II antimicrobials, a classification by the Netherlands Veterinary Medicines Authority (SDa). Type II antimicrobials have a high selection risk for ESBLs. The population attributable risk over one year was calculated by

$$PAF = \frac{\Pr\{D=1|t \leq 365\} - \Pr\{D=1|t \leq 365 \cap Z=0\}}{\Pr\{D=1|t \leq 365\}}$$
, with  $D$  being a binary value indicating a positive sample,  $t$  is the time of sampling, and  $Z$  indicates the risk factor. The probability  $\Pr$  was determined by integration of the empiric baseline hazard function.

## Conclusions

- A minor fraction of ESBL carriage could be attributed to antimicrobial usage in these cattle herds
- Feeding colostrum of cows treated with penicillin was not associated with ESBL carriage in the calf sampled between birth and two months of age
- The dynamics suggest repeated introduction and expansion of individual ESBL genes, but short persistence of ESBL genes
- The prevalence of carriage of ESBLs is mostly low on Dutch dairy farms, but some incidental high number of ESBL positive cows were detected.