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Seasonality of antimicrobial use in Dutch food-producing animals

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ABSTRACT

Due to globally increasing antimicrobial resistance (AMR), it is pivotal to understand factors contributing to antimicrobial use (AMU) to enable development and implementation of AMR-reducing interventions. Therefore, we explored seasonal variations of systemic AMU in food-producing animals in the Netherlands. Dutch surveillance data from January 2013 to December 2018 from cattle, pig, and broiler farms were used. AMU was expressed as the number of Defined Daily Dosages Animal per month (DDDA/animal-month) per farm by animal sector, antimicrobial line (first, second, and third), antimicrobial class, and farm type. Seasonality of AMU was analyzed using Generalized Additive Models (GAMs) with DDDA/animal-month as outcome variable, and year and month as independent variables. Year and month were modelled as smooth terms represented with penalized regression splines.Significant seasonality of AMU was found in the cattle and pig sectors, but not in broilers. Significant seasonality of AMU was found mainly for first-line antimicrobials. In the cattle sector, a significant increase during winter was found for the use of amphenicols (an increase of 23.8%) and long-acting macrolides (an increase of 3.4%). In the pig sector, seasonality of AMU was found for pleuromutilins ($p < 0.001$) with an increase of 20% in October-November. The seasonality of pleuromutilins was stronger in sows/piglets (an increase of 47%) than in fattening pigs (16% increase). Only in fattening pigs, the use of amphenicols showed a significant seasonality with an increase of 11% during winter ($P < 0.001$). AMU in cattle and pig sectors shows seasonal variations likely caused by seasonality of diseases. In broilers, no AMU seasonality was observed, possibly due to the controlled environment in Dutch farms. In the context of the one health concept, future studies are necessary to explore whether this seasonality is present in other populations and whether it has implications for antimicrobial resistance in humans through the food chain.

1. Introduction

Antimicrobials have been used in food-producing animals to prevent and treat diseases, and as growth promoters (Landers et al., 2012). Antimicrobials used in animals are the same as or closely related to those used in humans (Landers et al., 2012; Manyi-Loh et al., 2018). Antimicrobial use (AMU) in humans has been associated with antimicrobial resistance (AMR) (Goossens et al., 2005), but a direct link between AMU in food-producing animals and AMR in humans has not yet been conclusively established. Although some studies showed limited genetic similarities between resistant bacteria in livestock and in humans (de Been et al., 2014; Dorado-García et al., 2018; Mughini-Gras et al., 2019), transmission of clinically important resistant bacteria from animals to humans via the food-chain has been reported (Dierikx et al., 2010; Leverstein-van Hall et al., 2011; Dierikx et al., 2013; Voets et al., 2013). Therefore, in several countries, strategies have been implemented to

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reduce AMU in food-producing animals to decrease AMR in animals and in humans.

In the Netherlands, mandatory reduction targets were defined in 2008. Total AMU in food-producing animals should be reduced by 20% in 2011, by 50% in 2013 and by 70% in 2015, with 2009 as reference year (Mevius and Heederik, 2014). To attain this goal, a transparent monitoring and benchmarking system for AMU by farms and veterinarians was introduced, and use of clinically important antimicrobials for human medicine (e.g. 3rd and 4th generation cephalosporines and fluoroquinolones) was subject to restriction policies (Mevius and Heederik, 2014). As a result, the sales of antimicrobials to Dutch farms decreased by 63.4% from 2009 to 2017 (SDa, 2018), followed by a reduction of approximately 50% of antimicrobial resistance of *E. coli* in veal calves, pigs and broilers (Dorado-García et al., 2016). Although the trend over the years is closely monitored, little is known about seasonal variation within the year of AMU in food-producing animals.

Seasonal variation in AMU has been shown to be associated with differences in AMR between winter and summer seasons in wastewater (Gönder et al., 2021), indicating that insight in trends in seasonality of AMU for both humans and animals is also needed in approaches to decrease AMR. Seasonality of AMU has been described in humans and companion animals, and was attributed to peaks in seasonal infections. In humans, AMU was higher in winter than in summer, which was associated with respiratory infections at the community level (Goossens et al., 2005; Suda et al., 2014; Martinez et al., 2019). In contrast, in companion animals, AMU was higher in summer, which may be explained by allergic dermatitis seen in the warmer months (Hardefeldt et al., 2018; Hopman et al., 2019). Although a seasonal peak of infectious diseases has been described in food-producing animals (Carrique-Mas et al., 2010; Bahrndorff et al., 2013; Zhang et al., 2016), seasonality of AMU has not yet been reported. Since there are increasing trends of AMR, it is pivotal to understand factors contributing to AMR to enable development and implementation of antibiotic stewardship programs. Therefore, in this study we aimed to describe seasonal variations in the use of systemic antimicrobials in Dutch cattle, pig and broiler farms, using time series analyses.

2. Methods

2.1. Study design and data description

Longitudinal data from January 2013 to December 2018 were obtained from two databases of the Dutch Veterinary Medicines Institute (SDa): 1) the antimicrobial veterinary medical products (AVMPs) database (DG-standard database), containing information on all AVMPs authorized for sale in the Netherlands and conversion factors to treated kilograms per species; and 2) the delivery records of the amount of antimicrobials used on farms. The delivery records included information on the administration date, number of packages delivered, the antimicrobial class, and the number of animals present at the farm in a specific year. We used the delivery records from all cooperatives of farms in the broiler and cattle sector (excluding the veal farming sector). For the pig sector, one of the two co-operations of farms agreed to share data for this study, representing approximately 32% of the pig farms.

2.2. Calculation of antimicrobial use per month

Since 2011, the SDa has monitored AMU using both the DG-standard data set and the delivery records from farms in the monitored livestock sectors. The SDa reports AMU on a yearly basis as the number of Defined Daily Dosages Animal per year (DDDA/animal-year) to express the amount of antimicrobials used at a particular livestock farm (SDa, 2022). In the Netherlands, antimicrobials for veterinary use are exclusively prescribed by veterinarians, therefore the reported DDDA/animal-year reflects the total amount of antimicrobials used.

Because we aimed to describe seasonality of AMU while accounting for possible differences by antimicrobial line (first, second and third), antimicrobial class and farm type, we adapted the standardized operating procedure of the SDa. In order to avoid noise in the seasonal effect estimation, we excluded all farms that reported zero AMU and extreme values of AMU (lower than the 1st percentile and higher than the 99th percentile). The number of excluded farms varied per year and animal sector. On average, the percentage of excluded farm was 27% in the cattle sector, 20% in the pig sector and 30% in the broiler sector.

Afterwards, we calculated DDDA/animal on a monthly basis (DDDA/animal-month) instead of on a yearly basis. Antimicrobials were categorized as first, second and third line based on a report by the Dutch working party on Veterinary Antibiotic Policy (Table S1)(WVAB, 2018b), and farm types based on SDa yearly reports (SDa, 2022). We categorized the cattle sector into dairy and non-dairy, and the pig sector into sows/piglets and fattening pigs. The broiler sector was considered as one unique group including farms with conventional and non-conventional management systems.

For calculations, the DG-standard database and the delivery records per farm were merged by the European Article Number (EAN), which is a unique code for every AVMP. Afterwards, a three-step procedure was applied. In the first step, the merged database was grouped by year and month to determine the number of unique farms reporting antimicrobial use and to calculate the total animal weight per farm. The total animal weight was calculated by multiplying the total number of animals present at the farm in a year (assuming that this number is constant within a year) by standardized average body weights used by the SDa (Table S2).

In the second step, we grouped the dataset by year, month, type of farm, antimicrobial line and antimicrobial class. Using this grouping, we summed the total animal weight and the total of treated kilograms. The total of treated kilograms was estimated by multiplying the number of packages delivered to a specific farm by the treated kilograms animal per day for every AVMP. The treated kilograms per day expresses the amount of animal (per species) in kilograms that can be treated during 1 day with 1 unit (ml, g or piece) of a specific AVMP. The SDa calculates this based on the average authorized dosage of the active compound for every AVMP, considering also the duration of action of that specific AVMP product. The number of treated kilograms of a specific AVMP package always allows to treat the same amount of animal kilograms.

In the final step, again grouped by year, month, type of farm, antimicrobial line and antimicrobial class, we calculated the DDDA/animal-month per month from January 2013 to December 2018 by dividing the sum of the treated kilograms per farm by the sum of the total weight. The DDDA/animal-month represents, on average, the number of days per month that an animal kilogram in a farm was treated with an AVMP.

Calculations of DDDA/animal-month were done in R version 4.0.3 (R Core Team, 2020) and RStudio version 1.4.1106 using the tidyverse package. An example of the coding can be found in the supplementary material (Table S3).

2.3. Data analysis

Seasonality of AMU in the cattle, pig and broiler sectors was analyzed using generalized additive models (GAMs) which are suitable for time series data. A GAM is a generalized linear model that allows for non-linear associations, in which the response variable depends linearly on a number of smooth functions of independent variables. These smooth functions are splines and their sum form a GAM. The interpretation of GAM results relies on visualization of plots (Wood, 2017).

We used the same approach to fit all GAMs separately for each animal sector by antimicrobial choice, antimicrobial class, and farm type. In total, 48 GAMs were fitted for the cattle sector, 35 for the pig sector and 9 for the broiler sector. The outcome variable in the GAMs was the DDDA/animal-month, and the independent variables were year and month. Year and month were modelled as smooth terms to account for any trend over years (i.e., long-term change between years) and seasonality within years (i.e., change within a year) (Simpson, 2014).

Table 1
Average antimicrobial use (AMU) expressed in DDDA/animal-year in cattle, pig and broiler farming sectors by antimicrobial line, antimicrobial class and type of farm over the total study period from January 2013 to December 2018. Farms with zero AMU are excluded.

a) Cattle sector

Antimicrobials	Dairy cattle						Non-dairy cattle					
	2013	2014	2015	2016	2017	2018	2013	2014	2015	2016	2017	2018
	n	n	n	n	n	n	n	n	n	n	n	n
	17717	17504	17510	17276	16756	16196	6656	6430	6198	5939	5749	5444
First-line	**1.79**	**1.70**	**1.58**	**1.58**	**1.68**	**1.72**	**1.50**	**1.54**	**1.51 (80.32)**	**1.49**	**1.35**	**1.44**
(% of total AMU)	**(61.94)**	**(72.65)**	**(72.48)**	**(73.83)**	**(77.06)**	**(78.9)**	**(67.57)**	**(76.62)**		**(78.01)**	**(77.59)**	**(78.69)**
Amphenicols	0.03	0.04	0.04	0.04	0.03	0.03	0.19	0.19	0.19	0.19	0.16	0.17
Macrolides/ lincosamides	0.03	0.05	0.07	0.04	0.04	0.04	0.16	0.19	0.17	0.16	0.15	0.11
Penicillins	1.25	1.13	1.05	1.09	1.22	1.27	0.34	0.41	0.43	0.43	0.39	0.45
Pleuromutilins												
Tetracyclines	0.32	0.31	0.24	0.24	0.22	0.22	0.6	0.6	0.57	0.56	0.52	0.61
Trimethoprim/ Sulfonamides	0.16	0.17	0.18	0.17	0.17	0.17	0.21	0.15	0.15	0.16	0.13	0.10
Second-line	**1.09**	**0.64**	**0.60**	**0.56**	**0.50**	**0.46**	**0.62**	**0.46**	**0.37 (19.68)**	**0.42**	**0.39**	**0.38**
(% of total AMU)	**(37.72)**	**(27.35)**	**(27.52)**	**(26.17)**	**(22.94)**	**(21.1)**	**(27.93)**	**(22.89)**		**(21.99)**	**(22.41)**	**(20.77)**
Aminoglycosides	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.01	0.02	0.01
Aminopenicillins	0.34	0.28	0.28	0.26	0.24	0.22	0.12	0.13	0.13	0.11	0.11	0.13
Cephalosporins 1st/ 2nd gen.	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00
Combinations*	0.71	0.33	0.29	0.26	0.23	0.20	0.39	0.22	0.17	0.23	0.2	0.15
Long-acting Macrolides	0.01	0.01	0.01	0.01	0.01	0.01	0.06	0.07	0.03	0.04	0.04	0.07
Polymyxins	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00
Quinolones	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.02	0.01	0.01
Thrid-line	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.11**	**0.00**	**0.00 (0.00)**	**0.00**	**0.00**	**0.00**
(% of total AMU)	**(0.35)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(4.95)**	**(0.00)**		**(0.00)**	**(0.00)**	**(0.00)**
Cephalosporins 3rd/ 4th gen.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fluoroquinolones	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00
Total AMU	**2.89**	**2.34**	**2.18**	**2.14**	**2.18**	**2.18**	**2.22**	**2.01**	**1.88**	**1.91**	**1.74**	**1.83**

b) Pig sector

Antimicrobials	Sow/piglets						Fattening pigs					
	2013	2014	2015	2016	2017	2018	2013	2014	2015	2016	2017	2018
	n	n	n	n	n	n	n	n	n	n	n	n
	547	230	805	793	755	715	1095	1365	1359	1299	1236	1129
First-line	**6.09**	**6.45**	**7.64**	**7.75**	**7.72**	**8.30**	**6.44**	**4.96**	**5.02**	**5.76**	**5.19**	**5.49**
(% of total AMU)	**(65.27)**	**(67.61)**	**(64.75)**	**(65.79)**	**(64.93)**	**(69.05)**	**(91.74)**	**(94.84)**	**(95.8)**	**(96.97)**	**(94.36)**	**(96.83)**
Amphenicols	0.09	0.15	0.17	0.39	0.30	0.32	0.09	0.15	0.19	0.22	0.23	0.22
Macrolides/ Lincosamides	0.31	0.30	0.45	0.43	0.55	0.35	0.31	0.30	0.72	0.90	0.92	0.83
Penicillins	0.67	0.88	0.87	0.80	0.80	1.97	0.67	0.88	0.35	0.33	0.39	0.91
Pleuromutilins	0.07	0.07	0.06	0.05	0.08	0.10	0.07	0.07	0.07	0.08	0.08	0.07
Tetracyclines	3.10	3.51	4.35	4.35	4.46	4.21	3.10	3.51	3.21	3.72	3.10	3.05
Trimethoprim/ Sulfonamides	1.86	1.55	1.76	1.74	1.53	1.35	1.86	1.55	0.48	0.51	0.46	0.40
Second-line	**3.24**	**3.09**	**4.16**	**4.03**	**4.17**	**3.71**	**0.59 (8.4)**	**0.27**	**0.22**	**0.18**	**0.31**	**0.18**
(% of total AMU)	**(34.73)**	**(32.39)**	**(35.25)**	**(34.21)**	**(35.07)**	**(30.87)**		**(5.16)**	**(4.20)**	**(3.03)**	**(5.64)**	**(3.17)**
Aminoglycosides	0.00	0.02	0.01	0.01	0.01	0.04	0.00	0.02	0.00	-	0.00	0.00
Aminopenicillins	1.92	2.06	3.04	2.93	2.76	2.41	1.92	2.06	0.11	0.12	0.26	0.12
Cephalosporins 1st/ 2nd gen.	-	-	-	-	-	-	-	-	-	-	-	-
Combinations*	0.24	0.07	0.06	0.05	0.03	0.03	0.24	0.07	0.03	0.02	0.01	0.00
Long-acting Macrolides	0.48	0.32	0.31	0.53	0.78	0.51	0.48	0.32	0.01	0.01	0.01	0.01
Polymyxins	0.58	0.57	0.72	0.49	0.55	0.71	0.58	0.57	0.06	0.03	0.02	0.04
Quinolones	0.02	0.05	0.02	0.02	0.03	0.02	0.02	0.05	0.01	0.00	0.00	0.01
Thrid-line	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**-**	**0.00**	**-**
(% of total AMU)	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**	**(0.00)**		**(0.00)**	
Cephalosporins 3rd/ 4th gen.	-	-	-	-	-	-	-	-	-	-	-	-
Fluoroquinolones	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	-
Total AMU	**9.33**	**9.54**	**11.8**	**11.78**	**11.89**	**12.02**	**7.02**	**5.23**	**5.24**	**5.94**	**5.50**	**5.67**

c) Broiler sector

Antimicrobials	2013	2014	2015	2016	2017	2018
	n	n	n	n	n	n
	645	618	605	537	547	555
First-line	**7.29 (52.45)**	**6.22 (36.87)**	**5.07 (32.77)**	**4.02 (32.16)**	**3.57 (31.68)**	**3.42 (26.61)**
(% of total AMU)						
Amphenicols						
Macrolides/lincosamides	0.33	0.29	0.49	0.25	0.20	0.25

(*continued on next page*)

Table 1 (*continued*)

a) Cattle sector						
Penicillins	2.22	2.32	1.47	1.05	0.67	0.50
Pleuromutilins	0.00					
Tetracyclines	2.74	1.87	1.75	1.54	1.50	1.59
Trimethoprim/Sulfonamides	2.01	1.74	1.35	1.17	1.20	1.08
Second-line	**6.35 (45.68)**	**10.5 (62.24)**	**10.32 (66.71)**	**8.41 (67.28)**	**7.64 (67.79)**	**9.36 (72.84)**
(% of total AMU)						
Aminoglycosides	0.04	0.02	0.03	0.00	0.02	0.02
Aminopenicillins	4.40	8.18	6.87	6.54	5.61	6.43
Cephalosporins 1st/2nd gen.						
Combinations	0.29	0.05	0.05	0.04	0.01	0.02
Long-acting Macrolides						
Polymyxins	0.06	0.07	0.09	0.07	0.02	0.07
Quinolones	1.56	2.19	3.28	1.75	1.97	2.83
Thrid-line	**0.26**	**0.15**	**0.09**	**0.08**	**0.06**	**0.08**
(% of total AMU)	**(1.87)**	**(0.89)**	**(0.58)**	**(0.64)**	**(0.53)**	**(0.62)**
Cephalosporins 3rd/4th gen.						
Fluoroquinolones	0.26	0.15	0.09	0.08	0.06	0.08
Total AMU	**13.90**	**16.87**	**15.47**	**12.50**	**11.27**	**12.85**

0.00 means that AMU was below 0.005, - means no use was reported, *Combinations refers to combination preparations (multiple antibiotics from the same class or different classes).
n = number of unique farms reporting antibiotic use during the study period.

In all GAMs, the smooth terms were represented with penalized regression splines, which are splines that use a combination of linear and polynomial functions by splitting the data in different parts, between knots, to fit the data for each part separately. Thus, the number of knots determines the smoothness of the splines. The number of allowed knots is the basis dimension of the GAM (Wood, 2017). GAMs were fitted using a standard number of knots for each smooth term, and the appropriate degree of smoothness was estimated from the data by restricted maximum likelihood (REML). The month term was modelled as a 12-knot cyclic cubic spline, which is a spline that joins up at the end-points to ensure continuity between January and December (Simpson, 2014; Wood, 2017). The year term was modelled as a 10-knot thin-plate regression spline, which is a type of spline used to visualize complex predictor-response associations without having a prior knowledge of the functional form of the data (Wood, 2017). GAM plots were constructed to show significant seasonality of AMU and the relative change (%) of the mean AMU per livestock sector and antimicrobial class over the study period.

Diagnostics for the fitted GAMs were performed and corrections were applied when needed. First, because the data were time series, residuals were checked for autocorrelation by visualization of autocorrelation function (ACF) plots (Simpson, 2014). In those cases that the smooth terms could not account for the temporal structure in the data, an autoregressive correlation (AR1) term was included to account for the temporal dependency of observations (Simpson, 2014). Second, the basis dimensions used for the smooth terms were checked by testing for oversmoothing by considering whether the chosen number of knots on the smooth terms was not too low, and by visually checking standard diagnostic plots to check for normal distribution and equal variance (Wood, 2021). In those cases that the number of knots was too low, the number of knots was increased until a proper model was obtained. Diagnostics for GAM residuals are available in the supplementary material (Table S4, Figs. S1 and S2). In general, all models adequately account for autocorrelation and oversmoothing was not present.

Statistical analysis was done in R version 4.0.3 (R Core Team, 2020) and RStudio version 1.4.1106 using the packages nlme (version 3.1–152), mgcv (version 1.8–34) and ggplot2 (version 3.3.3). GAMs diagnostics was performed using the function "gam.check" available in the mgcv package. All statistical tests were two-sided with a significance level of 0.05. No correction for multiple testing was implemented.

3. Results

3.1. Antimicrobial use in Dutch food-producing animals

Data were available from an average number of 11,614 cattle farms, 944 pig farms and 585 broiler farms per year in the study period (2013–2018). In general, the AMU in all animal sectors decreased over time and first-line antimicrobials accounted for more than 60% of total AMU, except in the broiler sector where they accounted for on average 30% and second-line antimicrobials accounted for more than 60% (Table 1a, b and c).

The cattle sector reported the lowest average AMU over the 6 years. The average AMU was higher in dairy cattle (average of 2.31 DDDA/year over the 6 years) than in non-dairy cattle (1.93 DDDA/year over the 6 years). Penicillins were often used, especially in dairy cattle (Table 1a). In the pig sector, average AMU was higher in sows/piglets (average of 11.06 DDDA/year over the 6 years) than in fattening pigs (5.77 DDDA/year over the 6 years). In both sow/piglets and fattening pigs, tetracyclines were used most frequently with more than 3 DDDA/year (Table 1b).

The broiler sector reported an average of 13.81 DDDA/year, and the highest use of third-line antimicrobials among the livestock sectors included in this study (average of 0.12 DDDA/year). In this sector, the second-line antimicrobials accounted for more that 45% of the total average AMU, of which aminopenicillins were often used in an average of 6.33 DDDA/year (Table 1c).

3.2. Seasonality of AMU in food-producing animals

Significant seasonality of AMU was found for several antimicrobial classes in the cattle and pig sectors, but could not be demonstrated for any antimicrobial in the broiler sector (Table 2). In the cattle sector, the use of long-acting macrolides (gamithromycin, tulathromycin and tildipirosin) showed the strongest seasonality with an increase of 42.9% in December-January and a decrease of 17.0% in June-July ($p < 0.001$), compared to the average AMU for this antibiotic class. In the pig sector, pleuromutilin use increased by 20.1% in October-November and decreased by 29.3% in May-Jun ($p < 0.001$).

3.3. Seasonality of AMU in dairy cattle and non-dairy cattle

In dairy cattle, the use of all first-line antimicrobials showed a statistically significant seasonality (Table 3). Use of amphenicols showed a strong seasonal pattern ($p < 0.001$) with an increase of 23.8% in

Table 2
Smooth terms in generalized additive models (GAMs) analyzing the time trends and the seasonal pattern of AMU (DDDA/animal-month) in Dutch cattle, pig and broiler sectors, by antimicrobial choice and antimicrobial class from January 2013 to December 2018.

Antimicrobial	Cattle sector						Pig sector						Broiler sector		
	Year p-value	Month p-value	Estimated average DDDA /month	Relative change (%)[a]		Seasonal pattern	Year p-value	Month p-value	Estimated average DDDA /month	Relative change (%)		Seasonal patten	Year p-value	Month p-value	Estimated average DDDA /month
				Peak	Trough					Peak	Trough				
First-line	< 0.001	0.01	0.07	2.6	-3.5	↓Feb/Mar ↑Jun/Jul	< 0.001	0.11	0.21	NS	NS	NS	< 0.001	0.40	0.91
Amphenicols	< 0.001	< 0.001	0.04	7.3	-6.1	↓Jul/Aug ↑Dec/Jan	< 0.001	< 0.001	0.08	6.8	-8.1	↓Jul/Aug ↑Dec/Jan	-	-	-
Macrolides /Lincosamides	< 0.001	0.54	0.05	NS	NS	NS	< 0.001	0.36	0.34	NS	NS	NS	< 0.001	0.82	0.60
Penicillins	< 0.001	0.001	0.13	3.9	-7.6	↓Feb/Mar ↑Jun/Jul	< 0.001	0.001	0.05	15.8	-19.5	↓Feb/Mar ↑Jul/Aug	0.09	0.83	1.67
Pleuromutilins	-	-	-	-	-	-	< 0.001	< 0.001	0.21	20.1	-29.3	↓May/Jun ↑Oct/Nov	-	-	-
Tetracyclines	< 0.001	0.05	0.04	2.7	-4.5	↓Mar/Apr ↑Oct/Nov	< 0.001	0.49	0.39	NS	NS	NS	< 0.001	0.99	1.43
Trimethoprim /Sulfonamides	< 0.001	0.001	0.03	2.1	-2.0	↓Jul/Aug ↑Nov/Dec	< 0.001	0.001	0.20	5.0	-7.8	↓Aug/Sep ↑Feb/May	0.004	0.51	0.51
Second-line	< 0.001	0.34	0.05	NS	NS	NS	< 0.001	0.02	0.14	2.8	-2.1	↓Feb/Mar ↑Aug/Sep	< 0.001	0.16	2.03
Aminoglycosides	< 0.001	0.68	0.02	NS	NS	NS	-	-	-	-	-	-			
Aminopenicillins[b]	0.024	0.78	0.05	NS	NS	NS	< 0.001	0.03	0.17	2.2	-2.7	↓Feb/Mar ↑Aug/Sep	< 0.001	0.15	2.00
Cephalosporines 1st/2nd gen.	< 0.001	0.35	0.02	NS	NS	NS	-	-	-	-	-	-	-	-	-
Combinations*	0.001	0.19	0.07	NS	NS	NS	0.21	0.68	0.06	NS	NS	NS	-	-	-
Long-acting Macrolides	< 0.001	< 0.001	0.04	42.9	-17.0	↓Jun/Jul ↑Dec/Jan	< 0.001	0.27	0.16	NS	NS	NS	-	-	-
Polymyxins	< 0.001	0.16	0.03	NS	NS	NS	< 0.001	0.31	0.09	NS	NS	NS	-	-	-
Quinolones	-	-	-	-	-	-	-	-	-	-	-	-	< 0.001	0.96	2.26
Third-line	< 0.001	0.97	0.01	NS	NS	NS	-	-	-	-	-	-	0.23	0.60	0.73
Cephalosporines 3rd/4th gen.	< 0.001	0.62	0.01	NS	NS	NS	-	-	-	-	-	-	-	-	-
Fluoroquinolones	< 0.001	0.96	0.01	NS	NS	NS	-	-	-	-	-	-	< 0.001	0.90	0.73

Year p-values indicate whether time trends are statistically significant. Month p-values indicate whether seasonality is statistically significant.
[a] Percentage of relative change based on the highest peak and lowest trough compared with the average DDDA/animal-month.
[b] Year predictor was modelled as parametric term in the GAM.
This means that time series for these antimicrobials were not calculated, because no AMU was reported in either that animal sector or in a specific month; NS, non-significant seasonal effect.
*Combinations refers to combination preparations (multiple antibiotics from the same class or different classes).

Table 3
Smooth terms in generalized additive models (GAMs) analyzing the mean seasonal pattern of AMU (DDDA/animal-month) in cattle sector by farm type, antimicrobial choice and antimicrobial class from January 2013 to December 2018.

Antimicrobial	Dairy cattle						Non-dairy cattle					
	Year p-value	Month p-value	Estimated average DDDA /month	Relative change[a] (%)		Seasonal pattern	Year p-value	Month p-value	Estimated average DDDA /month	Relative change (%)		Seasonal pattern
				Peak	Trough					Peak	Trough	
First-line	< 0.001	0.003	0.066	4.9	-2.0	↓Feb/Mar ↑Jun/Jul	< 0.001	< 0.001	0.076	9.2	-9.2	↓Mar/Apr ↑Oct/Nov
Amphenicols[b]	< 0.001	< 0.001	0.024	23.8	-7.2	↓Jul/Aug ↑Dec/Jan	< 0.001	< 0.001	0.064	9.2	-9.5	↓Jun/Jul ↑Nov/Dec
Macrolides/ Lincosamides	< 0.001	< 0.001	0.041	3.6	-1.4	↑Jul/Aug ↓Dec/Jan	< 0.001	0.19	0.106	NS	NS	NS
Penicillins	< 0.001	< 0.001	0.135	3.8	-7.4	↓Feb/Mar ↑Jun/Jul	< 0.001	0.38	0.060	NS	NS	NS
Pleuromutilins	-	-	-	-	-	-	-	-	-	-	-	-
Tetracyclines	< 0.001	< 0.001	0.037	1.9	-3.5	↓Mar/Apr	< 0.001	< 0.001	0.102	27.6	-23.5	↓May/Jun ↑Dec/Jan
Trimethoprim/ Sulfonamides	< 0.001	0.001	0.027	2.0	-1.6	↓Mar/Apr ↑Oct/Nov	< 0.001	< 0.001	0.076	11.2	-1.9	↓Mar/Apr ↑Oct/Nov
							< 0.001					
Second-line	< 0.001	0.61	0.049	NS	NS	NS	< 0.001	0.01	0.047	5.0	-3.6	↓Apr/May ↑Nov/Jan
Aminoglycosides	< 0.001	0.28	0.017	NS	NS	NS	< 0.001	0.41	0.028	NS	NS	NS
Aminopenicillins	< 0.001	0.89	0.042	NS	NS	NS	< 0.001	0.29	0.035	NS	NS	NS
Cephalosporines 1st/2nd gen.	< 0.001	0.31	0.017	NS	NS	NS	0.02	0.35	0.031	NS	NS	NS
Combinations*	< 0.001	0.23	0.071	NS	NS	NS	< 0.001	< 0.001	0.048	15.5	-5.5	↓May/Jun ↑Sep/Oct
Long-acting Macrolides	< 0.001	< 0.001	0.032	48.7	-31.8	↓May/Jul ↑Dec/Jan	< 0.001	< 0.001	0.073	23.9	-10.5	↓Apr/Jun ↑Dec/Jan
Polymyxins	< 0.001	< 0.001	0.024	22.8	-5.8	↓May/Jun ↑Dec/Jan	< 0.001	0.02	0.032	32.5	-43.2	↓Ene/Feb ↑/Aug/Sep
Quinolones	-	-	-	-	-	-	-	-	-	-	-	-
Third-line[b]	< 0.001	0.94	0.014	NS	NS	NS	0.37	0.96	0.024	NS	NS	NS
Cephalosporines 3rd/4th gen.	< 0.001	0.65	0.014	NS	NS	NS	-	-	-	-	-	-
Fluoroquinolones[b]	< 0.001	0.87	0.014	NS	NS	NS	0.18	0.92	0.024	NS	NS	NS

Year p-values, represent significant and non-significant time trends. Month p-values, represents significant and non-significant seasonality.
[a] Percentage of relative change based on the highest peak and lowest trough compared with the average DDDA/animal-month.
[b] Year predictor was modelled as parametric term in the GAM.
Means that time series for these antimicrobials were not calculated due to either no AMU was reported in that animal sector or in a specific month; NS, non-significant seasonal effect.
*Combinations refers to combination preparations (multiple antibiotics from the same class or different classes).

December-January and a decrease of 7.2% in June-July (Fig. 1). Use of penicillins, trimethoprim/sulfonamides and tetracyclines was approximately 2.0–4.0% higher in summer-autumn, and approximately 2.0–7.0% lower in winter-spring. Of the second-line antimicrobials, use of long-acting macrolides showed the strongest seasonal pattern ($p < 0.001$) with an increase of 48.7% in December-January and a decrease of 31.8% in May-July (Fig. 1). Additionally, a significant seasonal pattern was observed for polymyxins ($p < 0.001$), with an increase of 22.8% during winter (Fig. 1).

In non-dairy cattle, the shape of the seasonality of AMU was similar to that of dairy cattle (Table 3). The use of amphenicols and tetracyclines showed a significant seasonality ($p < 0.001$) and was 9.2% higher in November-December and 27.6% in September-October. Notably, the use of polymyxins showed a clear seasonality ($p < 0.001$) with an increase of 32.5% in August-September and a decrease of 43.2% in February-March. (Fig. 2).

3.4. Seasonality of AMU in sows, piglets and fattening pigs

In sows/piglets, a significant seasonality of AMU was found for three first-line antimicrobials: penicillins ($p < 0.001$), pleuromutilins ($p = 0.01$) and trimethoprim/sulfonamides ($p = 0.01$) (Table 4). Use of pleuromutilins showed the strongest seasonality with an increase in use of 46.7% in October-November and a decrease of 44.3% in April-May. Among second-line antimicrobials, only aminopenicillins use showed a significant but weak seasonality peaking in July-September ($p = 0.01$) (Table 4 and Fig. 3).

In fattening pigs, a significant seasonality of AMU was found only for first-line antimicrobials (Table 4). Similar to sows/piglets, pleuromutilins showed the strongest seasonality ($p < 0.01$) with an increase of 15.8% in October-November and a decrease of 9.4% in May-June. The use of amphenicols and tetracyclines was higher in December-February than in July-August, with an increase of 10.6% in amphenicol use (Fig. 4).

3.5. Trends of AMU in Dutch animal sectors

GAM results showed that the total AMU for the majority of antimicrobials significantly decreased over time in all animal sectors (Fig. S3).

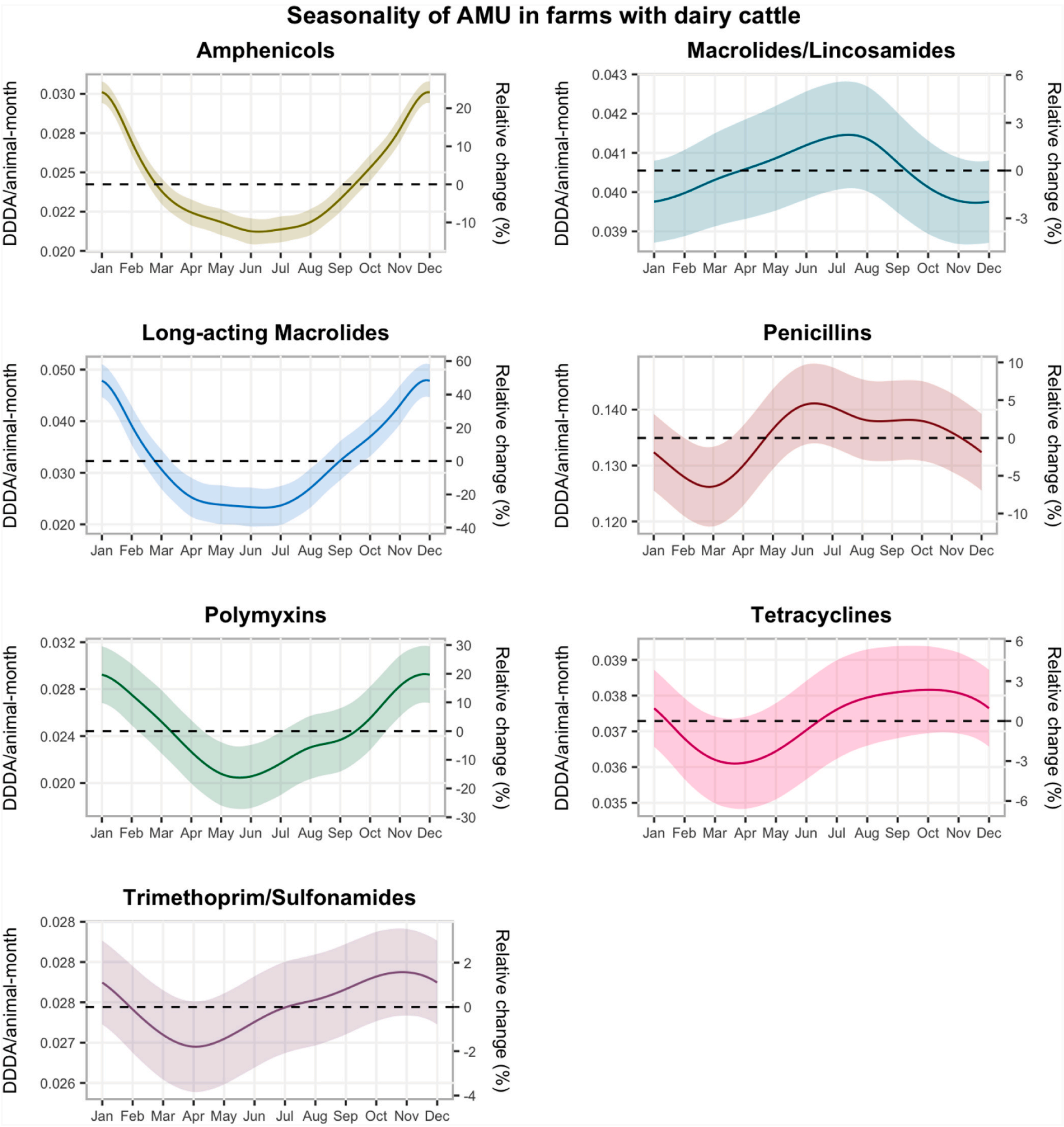

Fig. 1. GAM plot showing significant seasonality of AMU in dairy cattle from January 2013 to December 2018. The primary y-axis shows the mean of DDDA/animal-month per farm and the secondary y-axis shows the size of the seasonality as relative change compared with the average AMU (dashed lines) expressed in percentage. Seasonality of AMU was modelled using cyclic cubic splines (solid lines). GAMs included year as smooth terms to account for any long-term trend variation. Shadows around the splines show the 95% confidence intervals.

4. Discussion

In this study, we assessed seasonality of AMU in Dutch cattle, pig and broiler farming sectors using a time series analysis on surveillance data from January 2013 to December 2018. AMU was expressed as DDDA/animal-month per animal sector, farm type, antimicrobial class and antimicrobial line. The results of the GAM models showed significant seasonality of AMU for cattle and pig farms. Overall, the seasonality of AMU varied by antimicrobial class, but there was a small difference in seasonality between farm types. The use of the majority of antimicrobials increased in winter, with a range from 3% to 46% depending on antimicrobial class and farm type.

Seasonality in use was most frequently observed among first-line antimicrobials in both cattle and pig farms. More than 70% of total AMU consists of first-line antimicrobials, which are allowed to be used in Dutch farms for empirical treatment of diseases based on veterinarian advise (Mevius and Heederik, 2014). First-line antimicrobials have a wide range of indications specially for respiratory infections, enteric

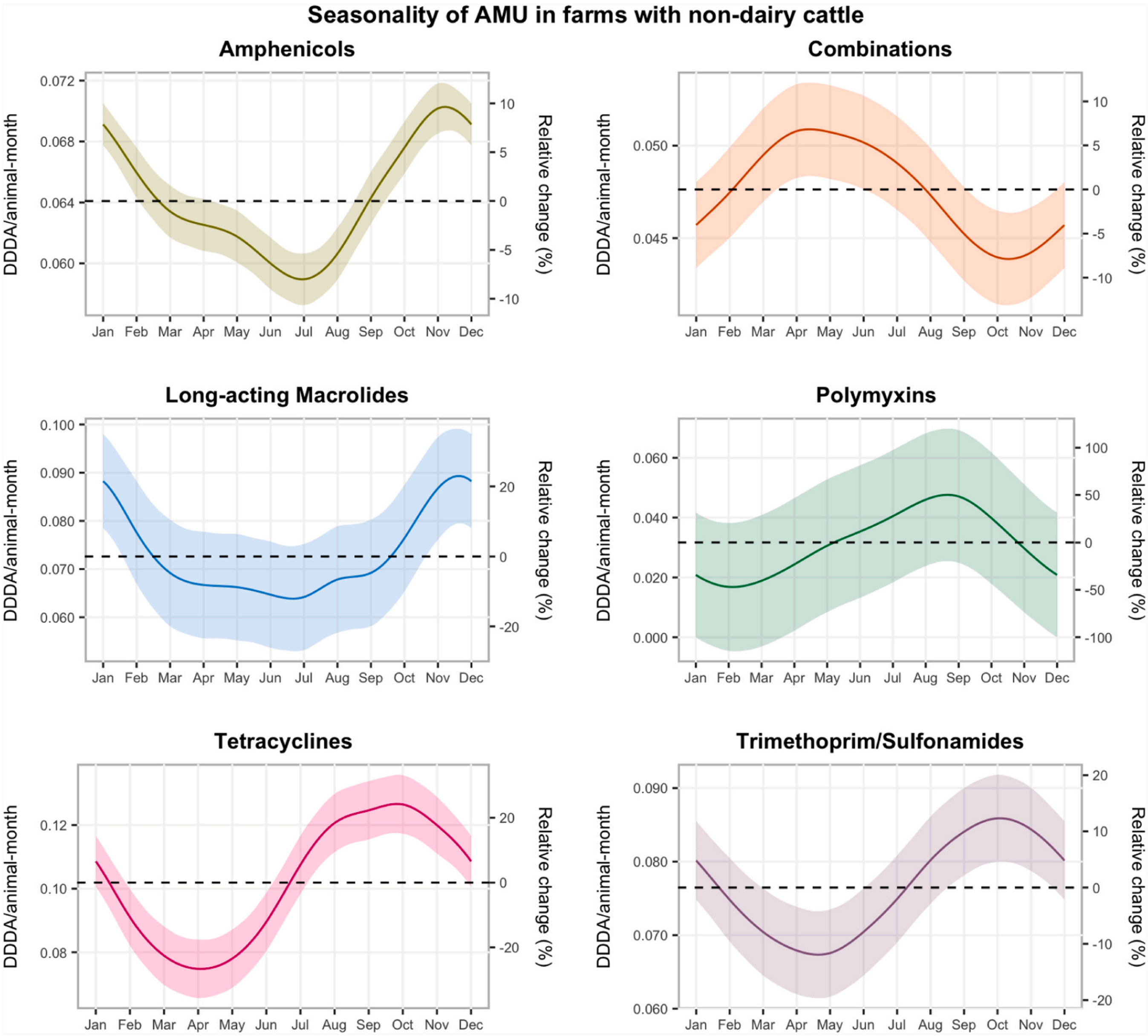

Fig. 2. GAM plot showing significant seasonality of AMU in non-dairy cattle from January 2013 to December 2018. The primary y-axis shows the mean of DDDA/animal-month per farm and the secondary y-axis shows the size of the seasonality as relative change compared with the average AMU (dashed lines) expressed in percentage. Seasonality of AMU was modelled using cyclic cubic splines (solid lines). GAMs included year as smooth terms to account for any long-term trend variation. Shadows around the splines show the 95% confidence intervals. Combinations refers to combination preparations (multiple antibiotics from the same class or different classes).

diseases, and skin problems (WVAB, 2016, 2017, 2018a). In cattle farms, we found an autumn-winter seasonality of trimethoprim/sulfonamides and tetracyclines, which are antibiotics to treat a variety of diseases (WVAB, 2016). The observed seasonality could partly be explained by the fact that trimethoprim/sulfonamides are the first choice antibiotics for enteric diseases caused by *Salmonella spp. and E. coli* (WVAB, 2016). A study in United Kingdom showed that *Salmonella* spp. infections increased during the second half of the year due to confinement indoors, intensive management, and calving season (Carrique-Mas et al., 2010); although, this has shown (still routinely monitored) not to be a problem in Dutch dairy cattle. In addition, trimethoprim/sulfonamides and tetracyclines are also used to treat respiratory infections, mastitis and claw problems, which are more prevalent in winter (Moosavi et al., 2014; Gaudino et al., 2022).

In sows and piglets, we found that the use of trimethoprim/sulfonamides increases in spring. These antibiotics are used as first option for neonatal diarrhea in Dutch farms (WVAB, 2018a). Possibly the found seasonality is in part due to the higher number of piglets born during spring (Wegner et al., 2014), although this is not a common phenomenon in Dutch pig husbandry. In fattening pigs, tetracyclines use increases in winter, that might be partly explained by an increase prevalence of respiratory infections in winter (Vangroenweghe and Thas, 2021).

Furthermore, winter peaks of long-acting macrolides and amphenicol use were observed in dairy cattle and in non-dairy cattle. It is known that respiratory infections occur mostly in winter, and these antimicrobial classes are used as first-line and second-line antimicrobials for respiratory diseases caused by *Pasteurella multocida* and *Mycoplasma spp.* in Dutch farms (WVAB, 2016, 2017). In contrast, a summer peak of macrolides/lincosamides use was found only in dairy cattle, just as with

Table 4
Smooth terms in generalized additive models (GAMs) analyzing the mean seasonal pattern of AMU (DDDA/month-year) in pig sector by farm type, antibiotic choice and antibiotic class from January 2013 to December 2018.

Antibiotic	Sows/piglets						Fattening pigs					
	Year p-value	Month p-value	Estimated average DDDA /month	Relative change[a] (%)		Seasonal effect	Year p-value	Month p-value	Estimated average DDDA /month	Relative change (%)		Seasonal effect
				Peak	Trough					Peak	Trough	
First-line	0.01	0.586	0.174	NS	NS	NS	< 0.001	0.001	0.261	3.4	-2.3	↓Jun/Jul ↑Dec/Jan
Amphenicols	< 0.001	0.887	0.064	NS	NS	NS	< 0.001	< 0.001	0.101	10.6	-12.1	↓Jun/Jul ↑Dec/Feb
Macrolides/ Lincosamides	-	-	-	-	-	-	< 0.001	0.70	0.404	NS	NS	NS
Penicillins	< 0.001	< 0.001	0.049	13.2	-19.7	↓Feb/Mar ↑Jul/Aug	< 0.001	0.02	0.055	10.1	-13.4	↓Dec/Feb
Pleuromutilins	< 0.001	0.01	0.198	46.7	-44.3	↓Mar/May ↑Sep/Oct	< 0.001	0.03	0.199	15.8	-9.4	↓Apr/May ↑Oct/Nov
Tetracyclines[b]	0.73	0.365	0.349	NS	NS	NS	< 0.001	0.002	0.424	3.8	-3.3	↓Jun/Jul ↑Dec/Jan
Trimethoprim /Sulfonamides	< 0.001	0.01	0.166	5.0	-4.7	↓Aug/Sep ↑Mar/Apr	< 0.001	0.50	0.400	NS	NS	NS
Second-line	< 0.001	0.01	0.142	2.4	-1.9	↓Dec/Feb ↑Jun/Jul	0.04	0.49	0.152	NS	NS	NS
Aminoglycosides	-	-	-	-	-	-	-	-	-	-	-	-
Aminopenicillins	< 0.001	0.01	0.167	3.0	-3.0	↓Feb/Mar ↑Jul/Aug	0.02	0.76	0.157	NS	NS	NS
Cephalosporines 1st/2nd gen.	-	-	-	-	-	-						
Combinations*[b]	0.73	0.738	0.047	NS	NS	NS	0.13	0.25	0.114	NS	NS	NS
Long-acting Macrolides	< 0.001	0.298	0.159	NS	NS	NS	-	-	-	-	-	-
Polymyxins	< 0.001	0.098	0.079	NS	NS	NS	< 0.001	0.83	0.149	NS	NS	NS
Quinolones	-	-	-	-	-	-	-	-	-	-	-	-
Third-line	-	-	-	-	-	-	-	-	-	-	-	-
Cephalosporines 3rd/4th gen.	-	-	-	-	-	-	-	-	-	-	-	-
Fluoroquinolones	-	-	-	-	-	-	-	-	-	-	-	-

Year p-values, represent significant and non-significant time trends. Month p-values, represents significant and non-significant seasonality.
[a] Percentage of relative change based on the highest peak and lowest trough compared with the average DDDA/animal-month.
[b] Year predictor was modelled as parametric term in the GAM.
Means that time series for these antimicrobials were not calculated due to either no AMU was reported in that animal sector or in a specific month; NS, non-significant seasonal effect.
*Combinations refers to combination preparations (multiple antibiotics from the same class or different classes).

penicillins use, possibly because tylosin is authorized for, and recommended as first choice antibiotic for parenteral administration to treat subclinical mastitis (grade 1 and 2), and it is known that the incidence of mastitis caused by *Streptococcus uberis* and *E. coli* is higher in summer-autumn months (Olde Riekerink et al., 2007; WVAB, 2016).

Another important result was the significant seasonality of polymyxin use, despite the low overall use in Dutch cattle farms. In the Netherlands, the use of colistin in food-producing animals is restricted as a last option within second-line antimicrobials for oral administration for gastrointestinal *E. coli* infections (WVAB, 2016, 2018a). We found that the use of polymyxins increased during autumn and winter, possibly due to a higher circulation of multidrug-resistant *E. coli* in these months, when animals are kept indoors. As polymyxins are critically important antimicrobials for human medicine, further studies are needed to understand the meaning of this seasonality and its possible consequences for animal and human AMR.

The most pronounced seasonality of AMU in pig farms was found for pleuromutilins, with an autumn peak in sows and piglets. This finding is in agreement with a study in Canadian pig farms, where the higher use of tiamulin in autumn was associated with dysentery outbreaks caused by *Brachyspira hyodysenteriae* (Walczak et al., 2017). In Dutch pig farms, tiamulin is the first-line antimicrobial for both dysentery and respiratory infections (WVAB, 2018a). A study in Belgian and Dutch intensive pig farms showed that respiratory infections caused by *Mycoplasma hyopneumoniae* can be seen in piglets of 3–5 weeks with a higher risk during autumn (Vangroenweghe et al., 2015). Currently, the use of pleuromutilins is not limited to animals anymore (levamulin was introduced for human treatment in 2020), but resistance levels to these antimicrobials are still low, although a number of studies reported a decreased susceptibility in *B. hyodysenteriae*, indicating a possible impact on swine production due to the limited options to treat swine dysentery, which is a disease that causes a high mortality rate, impaired growth and high costs (van Duijkeren et al., 2014).

In general, the seasonality of AMU observed in pig farms showed more variability compared to cattle farms, reflected by wider confidence intervals around the seasonal effect. The reason behind this result is unknown, but possible explanations could include the variability of farm types (closed farms, production farms for piglets, farms with all-in-all-out), higher average number of pigs per farm due to a decrease in the number of farms (CBS, 2020), and the variation in the number of pigs by farming system and production cycle (Vangroenweghe et al., 2015). In contrast, in dairy cattle the number of animals is more stable within a year as dairy farms are closed farms without typical cycles.

In this study, we could not demonstrate seasonality of AMU in broiler farms. Likely, the controlled nature of the environment of Dutch broiler farms with all-in-all-out systems could limit seasonal variation of diseases and therefore the seasonality of AMU (Caekebeke et al., 2020). Also, broiler farms have been moving from a conventional production system to production of alternative, slow-growing breeds since 2017, and a change of production system (like fewer animals per square meter and egg hatching in the stable) could also lead to changes of AMU.

The seasonality of AMU we found in food-producing animals was

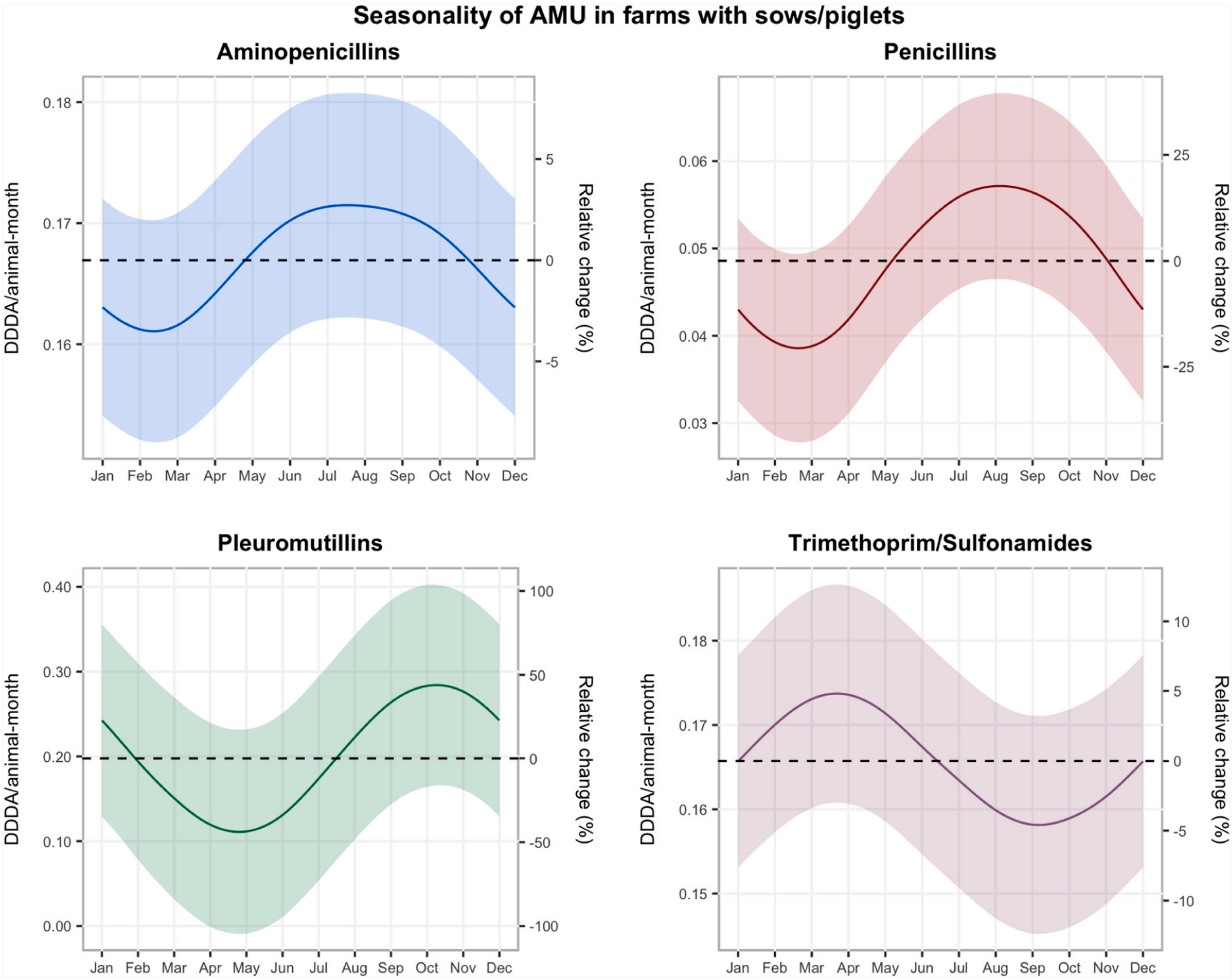

Fig. 3. GAM plot showing significant seasonality of AMU in sows and piglets from January 2013 to December 2018. The primary y-axis shows the mean of DDDA/animal-month per farm and the secondary y-axis shows the size of the seasonality as relative change compared with the average AMU (dashed lines) expressed in percentage. Seasonality of AMU was modelled using cyclic cubic splines (solid lines). GAMs included year as smooth terms to account for any long-term trend variation. Shadows around the splines show the 95% confidence intervals.

different from the one reported in companion animals (higher in summer) (Hardefeldt et al., 2018; Hopman et al., 2019), but similar to the seasonality of AMU in humans (higher in winter) (Goossens et al., 2005; Suda et al., 2014). As increased AMU is associated with increased AMR in food-producing animals (Manyi-Loh et al., 2018), further studies are needed to show if there are seasonal patterns in AMR in food-producing animals. Moreover, previous studies have described the possibility of AMR transmission from animals to humans through the food chain (Dierikx et al., 2010; Leverstein-van Hall et al., 2011; Dierikx et al., 2013; Voets et al., 2013), and hypothetically, seasonal patterns in AMU and AMR in food-producing animals might be associated with seasonal patterns in AMR in humans. This is in line with the one health concept and needs to be studied further. For example, studies could try to link seasonality of AMR in humans with AMU and AMR in animals in the same region and timeframe by using multiple time series analysis.

Our study has a number of strengths but also some limitations. To our knowledge, this is the first study describing seasonality of AMU in food-producing animals. We used routinely collected surveillance data that expresses the real amount of antimicrobials used in Dutch farms. We use data from a high number of farms covering the cattle, pig and broiler livestock sectors that reported AMU. One possible limitation is that the number of animals present at the farms is only available on a yearly basis; thus, seasonality in AMU due to fluctuations in farm size could go unnoticed or in fact, this fluctuation in farm size could create seasonality of AMU. The effect of this issue is limited, however, as it would only influence our results if all farms within an animal sector had fluctuated simultaneously and seasonally in farm size. Fluctuations in farm size could happen when a large proportion of the animals are transferred from farms to slaughterhouses. Based on European Statistical information (Eurostat), it is unlikely that the number of animals slaughtered influenced our results (See Table S6 for extra detail). Another possible limitation is that we could not analyze all type of farms separately, because, for some groups there was insufficient data to create the time series. For example, beef cattle farms, rearing farms, and suckler cow farms were analyzed together as non-dairy cattle. This may lead us to miss group-specific seasonality of AMU, however, we still found significant seasonal variation of AMU in the non-dairy cattle category. Finally, despite that we used different methodology and inclusion criteria for farms compared with the SDa (SDa, 2022), similar decreasing trends of AMU were observed due to the restrictive policies endorsed in the Netherlands. This may limit the generalization of the seasonality in AMU found in our study compared to the seasonality of AMU in high-use countries. However, we do not expect a large effect since the GAM models applied in our study were adjusted for any change in trend

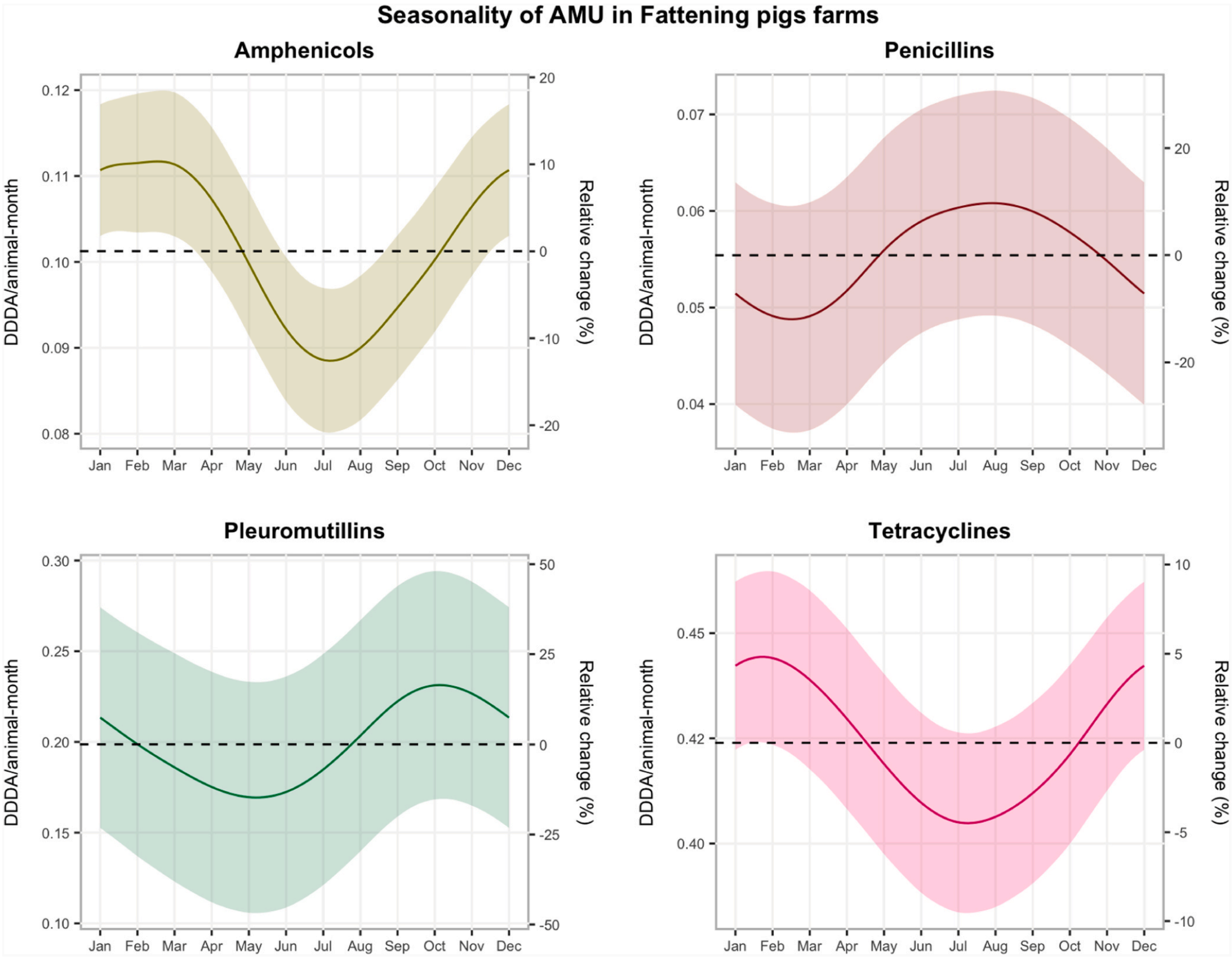

Fig. 4. GAM plot showing significant seasonality of AMU in fattening pigs from January 2013 to December 2018. The primary y-axis shows the mean of DDDA/animal-month per farm and the secondary y-axis shows the size of the seasonality as relative change compared with the average AMU (dashed lines) expressed in percentage. Seasonality of AMU was modelled using cyclic cubic splines (solid lines). GAMs included year as smooth terms to account for any long-term trend variation. Shadows around the splines show the 95% confidence intervals.

between years.

In conclusion, significant seasonality of AMU in food-animals was found predominantly among first-line antimicrobials in cattle and pig farms. The seasonality of AMU is likely to be caused by seasonality of animal diseases. The seasonality of diseases might be associated with production systems, managing practices, production cycles and farm size. Further studies are necessary to replicate our findings in independent animal populations, and to assess how all these determinants could influence the seasonality of AMU, and subsequent consequences for AMR in food producing animals and the potential transmission to humans trough the food chain.

Declaration of Competing Interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2023.106006.

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