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Antimicrobial Resistance in commensal *Escherichia coli* from livestock in Belgium: *Trend Analysis 2011-2018*

EXECUTIVE SUMMARY

Background & objective
Belgian trend analysis of antimicrobial resistance in faecal *Escherichia coli* (*E. coli*) retrieved from livestock during seven consecutive years (2011-2018) was performed in accordance with the European legislation.

Methodology
Samples collected by the Federal Agency for the Safety of the Food Chain (FASFC) were taken at the slaughterhouse for veal calves, broilers and fattening pigs and on farms for young beef cattle. *S*usceptibility was tested over consecutive years for 11±3 antimicrobial agents by a micro-dilution technique (Trek Diagnostics) and conversion of minimal inhibitory concentrations to binary qualitative values (Resistant/Susceptible) was done by means of the Epidemiological cut-offs values (ECOFFs) as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Statistics were carried out using SAS 9.3 software and R freeware.

For each animal category and year, the proportion of resistant isolates (p) was calculated for the individual antimicrobial agents and 95% confidence intervals (CI) were constructed for *logit(p)* to avoid interval boundaries outside the range [0-1]. Several statistical methods were used to model the probability of an isolate to be resistant: logistic regression models (in the univariate model each antimicrobial was considered separately), a linear Generalized Estimating Equations model (GEE) and non-linear mixed models (both multivariate models, taking into account the possible correlation between antimicrobial substances in a single model).
Similarly, multi-resistance (resistance to at least three antimicrobial families) was calculated and logistic regression models identified significant trends. Finally, a diversity index (weighted entropy) was calculated to describe the degree of diversity of multi-resistance.

Results
In **veal calves**, increase in prevalence of resistance was observed in 2018 compared to 2017 for all tested antimicrobials except azithromycin, gentamicin and meropenem. Despite high levels of resistance (>50%) for the eight consecutive years for AMP,SMX, TET, a rise of 11.09%% for AMP, 12.16% for SMX and 9.84% for TET between 2017 and 2018 were observed, which are statistically significant.
The linear multivariate model (GEE) showed a statistically significant decrease of resistance over time for AMP, TET, TAZ, FOT and GEN.
Based on the non-linear mixed multivariate model, when comparing to the year before, a constant significant decrease in resistance (odds ratio (OR<1) for all substances from 2012 to 2014 is noticed. However, as OR have been increasing since 2011 to 2018, this decrease in resistance prevalence was no more significant for any substance since 2017. Some substances also presented recent significant increase in resistance compared to the year before (AMP, FOT, SMX, TAZ, TMP, CH, TET).
Globally, lower prevalence of resistance was observed in *E. coli* from young **beef cattle** compared to veal calves, yet the same substances were involved: AMP, SMX, TET and TMP.

Between 2017 and 2018, prevalence increased for SMX (+18.56%), TMP (+6.34%). In two critical substances identified by the World Health Organization (CIP and FOT), resistance increased in 2017 (>5%, compared to 2016) but decreased in 2018 (-5.22% for CIP) or remained stable (+0.05% for FOT).

Based on the results of the linear multivariate model (GEE), the probability to be resistant significantly decreased over the 8 years for AMP, NAL, CIP and COL.

Based on the non-linear mixed multivariate model, when compared to the year before, there were constant significant decreases in resistance (OR<1) for FOT (2012 and 2013), AMP, TAZ, TMP (2012 to 2014) and CIP, NAL and TET (2012 to 2015). However, OR progressively increased and resistance became significant different compared to the previous year for SMX, TAZ since 2016 and TMP since 2018. COL resistance level continuously decreased (OR<1 since 2014) and became significantly lower compared to the year before since 2015.

In **broiler chickens,** a high prevalence of resistance (≥ 50%) was observed during the eight consecutive years for AMP, CIP and SMX. Prevalence of resistance was observed with values ≥ 50% for seven years for NAL and TMP. Prevalences of resistance increased by 9.95% and by 8.63% for FOT and for TAZ respectively from 2016 to 2017 then slightly decreased in 2018 (-0.92% and -2.38% respectively). However, increases by 14.56% for SMX, by 8.04% for AMP and by 11.28% for TMP were observed in 2018.

In **fattening pigs**, the prevalence of resistance for AMP, SMX, TET, TMP remained above 40% during seven years. Prevalence of FOT and TAZ increased between 2016 and 2017 but remained stable in 2018. However, the prevalence of resistance to SMX increased by 10.36% in 2018.

In all the tested samples, the proportion of **multi-resistant strains** (= strains resistant to at least three antimicrobials) was very high during the eight consecutive years for broiler chickens (>62%) and high for veal calves (>50%). In chickens and veal calves, prevalence of multi-resistance increased by 10% and 12.70% between 2017 and 2018 respectively. Compared to 2017, the proportion of fully susceptible strains decreased respectively by 13%, 23%, 4% and 3% in meat calves, young bovine, chickens, and pigs. Regarding weighted entropy, a 24% decrease is observed in 2018 in beef cattle. That means strains are more resistant to the same family of antimicrobials.

1. CONTEXT

This report summarises the results of the trend analysis of the data related to antimicrobial resistance in *Escherichia coli* (*E. coli*) during eight consecutive years (2011-2018) regarding commensal intestinal flora of several livestock categories in Belgium:

- Veal calves
- Young beef cattle
- Fattening pigs
- Broiler chickens

Commensal *E. coli* is regarded as a general indicator for resistance amongst Gram-negative bacteria. It can be frequently isolated from all animal species and is therefore suitable for comparisons and pertinent as target of surveillance programmes. Earlier studies have shown that the aforementioned livestock categories undergo a substantial antimicrobial selection pressure in Belgium (Filippitzi M. E. et al., 2017).

During sampling, faecal material was taken in the slaughterhouse or directly in the farms depending on the animal category. *E. coli* isolated and thereafter tested for its susceptibility to a panel of several antimicrobials.

The objectives of this study were two-fold:

- To provide a **trend analysis of the prevalence of resistant strains** over the eight consecutive years, the results were described and then statistically analysed to check whether the observed trends (increase or decrease) were statistically significant.

- To evaluate the level of **multi-resistance and its trend** over the same period: using the same data, the proportion of multi-resistant strains was calculated in each animal category (*i.e.* resistance to more than two antimicrobials (= at least three) in the same strain) and checked whether there was a significant trend.

2. MATERIAL AND METHODS

- **A. Sampling**

Samples of fresh faeces were collected each year by agents of the Federal Agency for the Safety of the Food Chain (FASFC) according to standardized technical sampling instructions (PRI codes) as part of a nationwide surveillance programme.

Samples were taken from the following categories of food-producing animals:

- **Veal calves**: calves kept in specialized units for fattening and slaughtered at an average age of 8 months. In 2011, faecal samples were taken on the floor at the farm level (PRI-516: 10 animals/farm of 7 months or younger), while after 2011 the samples were taken directly from the rectum of the animals at the slaughterhouse (PRI-036: 1 animal sampled/farm)
- **Beef Cattle** (meat production): young animals (7 months or younger) from farms raising beef cattle for meat production. Faecal samples were taken from the floor at the farm (PRI-515: 1 sample consisted of a pool of faeces collected from different spots on the floor representing at least 10 animals).
- **Broiler chickens**: samples were taken at the slaughter house (PRI-019: pools of pairs of caeca from 10 chickens /batch)
- **Fattening pigs**: faecal samples of fattening pigs older than 3 months were taken from the rectum at the slaughterhouse (PRI-035: 1 animal /origin farm).

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Following EFSA's recommendations, the target sample size for each animal category was fixed to 170 isolates. (European Food Safety Authority (EFSA), 2008b),

In order to improve representativeness, the sampling was stratified by province proportionally to the number of registered herds or slaughterhouses.

- **B. Isolation of the strains and antimicrobial susceptibility testing**

Isolates of *E. coli* strains were obtained from the faecal samples. The isolations were performed by ARSIA except for broiler chickens (PRI019), as of August 2017, analyses were performed at the laboratories of the Federal Agency for the Safety of the Food Chain at Melle and Gembloux, according to the standard operating procedures (SOP). The isolates were sent to the National Reference Laboratory (Sciensano) for bacterial species confirmation and antimicrobial susceptibility testing*. S*usceptibility was tested by a micro-dilution technique (Trek Diagnostics) as it is described in the annual reports. The antimicrobials common to the seven years (2011-2018) and those tested from 2014 to 2018 are presented in **Table 1**. For each strain and each antimicrobial substance, the Minimal Inhibitory Concentration (MIC) was recorded: MIC is defined as the lowest concentration by which no visible growth could be detected. MICs were semi-automatically recorded and stored in a database (**Annexe 1)**.

Table 1. In E.Coli, panel of antimicrobials tested, in black: form 2011 to 2018, in green: from 2014 to 2018

Symbol	Antimicrobial
AMP	Ampicillin
AZI	Azithromycin
CHL	Chloramphenicol
CIP	Ciprofloxacin
COL	Colistin
FOT	Cefotaxime
GEN	Gentamicin
MER	Meropenem
NAL	Nalidixic acid
SMX	Sulphamethoxazole
TAZ	Ceftazidime
TET	Tetracycline
TIG	Tigecyclin
TMP	Trimethoprim

- **C. DATA**

The datasets for 2011-2018 were formatted in Excel files by the Department of Bacteriology of Sciensano and validated by the FASFC. They included identification of the samples corresponding to each isolate recorded in the Laboratory Information Management System (LIMS) merged with the corresponding MIC value for each tested antibiotic. After several steps of cross-checking and cleaning of the data, seven yearly data sets were produced, imported, validated and analysed in SAS 9.3 software and R freeware. Emphasis was put on verifying that the animal category of the sample was correct. The final annual datasets contained the following fields: i. isolate identification number, ii. animal category, iii. sampling date and iiii. MIC values for each of the tested antimicrobials (µg/mL).

- **D. STATISTICAL METHODS**

All subsequent statistics were carried out using SAS 9.3 software and R freeware.

1. Prevalence

Quantitative MIC values were converted into binary qualitative values (Resistant/Susceptible) based on the susceptibility breakpoints defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)(European Committee on antimicrobial susceptibility testing). The ECOFFs (Epidemiological cut-offs values) were used in order to define strains as Resistant (R) or Susceptible (S) (**Annexe 1**).

For each animal category and year, the proportion of resistant isolates (p) was calculated per tested antimicrobial (resistance prevalence), as well as the associated 95% confidence interval (CI). In order to avoid interval boundaries outside 0-1, CI were constructed for *logit*(p).

2. Trend Analysis

The trends analysis aims at finding models to describe the variation of antimicrobial resistance over the years and to check if any change in resistance proportion was significant or not. For the antimicrobials used over the eight years period, several statistical methods were used to model the probability of an isolate to be resistant: logistic regression models (in the univariate model each antimicrobial was considered separately), a linear Generalized Estimating Equations model (GEE) and a non-linear mixed model (both multivariate models) taking into account the possible correlation between antimicrobial substances in a single model; assuming an unstructured correlation matrix in the GEE).

The results are shown as Odds Ratio (OR), where an OR higher than 1 means that the probability of resistance statistically significantly increases over time. Plots representing the log odds for each year were also produced for each antimicrobial and animal category. The odds represent the ratio of the probability to be resistant to the probability to be susceptible.
In this study, the effects of the different antimicrobials were assessed on an individual level. Hence, the 5% significance levels were specified for each antimicrobial separately. In order to adjust the p-values and reduce the chances of obtaining false-positive results (type I errors; i.e. detection of a trend when in reality there is no trend) when several dependent or independent statistical tests are being performed simultaneously on a single data set, both the Bonferroni's correction method and the linear step-up method of Benjamini and Hochberg (1995) (Benjamini Y. and Hochberg Y., 1995) were applied to the GEE (linear multivariate model). The resulting corrected p-values were produced and presented in annex.

3. Multi-resistance

Considering multi-resistance was considered in this report as resistance by an isolate to at least three antimicrobials belonging to any three antimicrobial families as recommended by EFSA (European Food Safety Authority (EFSA), 2014, European Food Safety Authority (EFSA), 2008a). Considering the antimicrobials common to the eight years, 11 antibiotics belonging to 9 different classes were considered in the analyses.
Based on this, for each animal category, the prevalence of multi-resistant isolates was calculated together with the 95% CI, considering resistance follows a normal distribution.
In addition, logistic regression models were used to check whether there was a significant trend over the years regarding the prevalence of multi-resistant strains, in each animal category.

In addition, a diversity index was calculated for multi-resistance: the weighted entropy.
This index ([0-1]) is calculated using R software based on the formula of Guiasu (Guiaşu S., 1971), to describe the degree of diversity of multi-resistance. A weighted entropy index close to 1 reflects a shift to multi-resistance to a greater number of antibiotics. This latter index was calculated.

4. RESULTS

A. Prevalence

Table 2. Prevalence of resistance by antimicrobial substance, by animal category and by year.

Prevalence of resistance per species, per antimicrobial and per year (number of samples, mean, low and high confidence intervals (%))

		2011				2012				2013				2014			
Veal calves	AMP	34	70.59	52.45	83.93	181	74.03	67.09	79.95	202	64.36	57.46	70.71	188	54.79	47.56	61.82
	AZI		/	/	/		/	/	/		/	/	/		3.19	1.43	6.97
	CHL		50.00	33.00	67.00		42.54	35.48	49.92		33.66	27.44	40.52		25.53	19.76	32.31
	CIP		41.18	25.42	58.98		43.09	36.01	50.47		27.36	21.60	34.00		22.34	16.91	28.91
	COL		14.71	5.96	31.91		6.08	33.78	10.69		5.94	3.39	10.21		2.66	1.10	6.28
	FOT		0.00	/	/		9.94	6.33	15.29		3.47	1.65	7.13		0.53	0.07	3.74
	GEN		20.59	9.75	38.37		6.63	37.83	11.37		7.92	4.89	12.59		5.85	3.25	10.31
	MER														0.00	/	/
	NAL		41.18	25.42	58.98		38.12	31.28	45.47		27.72	21.94	34.35		20.74	15.50	27.20
	SMX		79.41	61.63	90.25		75.14	68.26	80.94		70.30	63.58	76.24		57.45	50.21	64.38
	TAZ		0.00	/	/		11.05	7.21	16.57		3.96	1.98	7.76		0.53	0.07	3.74
	TET		73.53	55.45	86.11		79.01	72.39	84.37		76.73	70.35	82.09		68.09	61.02	74.41
	TIG														0.00	/	/
	TMP		70.59	52.45	83.93		69.61	62.46	75.93		57.92	50.94	64.59		51.06	43.88	58.20
Beef cattle	AMP	154	25.32	19.02	32.87	175	35.43	28.64	42.86	204	19.12	14.25	25.15	164	20.73	15.15	27.69
	AZI		/	/	/		/	/	/		/	/	/		0.61	0.08	4.28
	CHL		14.29	9.55	20.83		17.71	12.70	24.16		16.67	12.12	22.48		15.85	10.98	22.34
	CIP		11.04	6.94	17.12		18.29	13.19	24.79		8.82	5.61	13.62		8.54	5.09	13.97
	COL		0.65	0.09	4.56		2.86	1.18	6.73		1.47	0.47	4.50		0.61	0.08	4.28
	FOT		4.55	2.16	9.30		6.29	3.49	11.05		3.43	1.63	7.06		2.44	0.91	6.38
	GEN		2.60	0.97	6.79		4.00	1.9	8.21		6.86	4.09	11.30		4.88	2.44	9.51
	MER		/	/	/		/	/	/		/	/	/		0.00	/	/
	NAL		11.69	7.45	17.87		17.14	12.21	23.53		8.82	5.61	13.62		7.32	4.18	12.51
	SMX		30.52	23.69	38.32		42.29	35.12	49.80		32.84	26.70	39.64		23.78	17.83	30.97
	TAZ		3.90	1.74	8.47		7.43	4.34	12.43		2.45	1.02	5.79		2.44	0.91	6.38
	TET		19.48	13.92	26.58		36.00	29.17	43.45		21.57	16.42	27.80		18.29	13.05	25.04
	TIG		/	/	/		/	/	/		/	/	/		0.00	/	/
	TMP		19.48	13.92	26.58		28.57	22.31	35.78		20.59	15.55	26.74		15.24	10.47	21.66
Chickens	AMP	420	84.76	80.98	87.90	320	81.56	76.90	85.46	234	84.62	79.36	88.72	158	72.78	65.24	79.21
	AZI		/	/	/		/	/	/		/	/	/		4.43	2.11	9.07
	CHL		24.29	19.41	28.64		45.94	40.52	51.45		32.48	26.75	38.79		20.89	15.19	28.01
	CIP		62.86	58.11	67.37		79.06	74.23	83.19		74.79	68.78	79.97		69.62	61.93	76.35
	COL		0.48	0.12	1.89		4.69	2.84	7.65		1.71	0.64	4.50		0.00	/	/
	FOT		19.05	15.56	23.11		29.38	24.62	34.63		10.26	6.95	14.89		8.86	5.29	14.48
	GEN		4.05	2.53	6.43		6.25	4.06	9.51		5.13	2.92	8.85		5.70	2.97	10.65
	MER		/	/	/		/	/	/		/	/	/		0.00	/	/
	NAL		62.86	58.11	67.37		78.44	73.57	82.62		70.09	63.86	75.64		63.29	55.42	70.51
	SMX		75.00	70.62	78.92		81.25	76.57	85.18		69.23	62.98	74.85		58.23	50.31	65.74
	TAZ		17.14	13.82	21.07		25.94	21.41	31.05		10.68	7.30	15.37		7.59	4.34	12.97
	TET		64.76	60.05	69.20		70.63	65.37	75.38		59.83	53.37	65.96		45.57	37.89	53.47
	TIG		/	/	/		/	/	/		/	/	/		0.00	/	/
	TMP		63.10	58.35	67.60		70.22	64.94	75.01		60.26	53.80	66.37		49.37	41.57	57.20
Pigs	AMP	157	49.04	41.23	56.91	217	47.47	40.85	57.17	206	45.15	38.43	52.05	184	41.30	34.35	48.62
	AZI		/	/	/		/	/	/		/	/	/		1.09	0.27	4.29
	CHL		26.75	20.35	34.30		28.57	22.91	34.99		26.21	20.62	32.70		28.80	22.67	35.83
	CIP		14.10	9.43	20.57		13.36	9.42	18.62		5.83	3.32	10.02		2.17	0.81	5.70
	COL		0.64	0.09	4.47		0.92	0.23	3.65		2.43	1.01	5.74		0.54	0.08	3.82
	FOT		4.46	2.12	9.12		2.76	1.24	6.05		0.97	0.24	3.84		0.54	0.08	3.82
	GEN		3.82	1.71	8.31		0.92	0.23	3.65		1.94	0.72	5.10		2.17	0.81	5.70
	MER		/	/	/		/	/	/		/	/	/		0.00	/	/
	NAL		11.46	7.31	17.54		12.90	9.03	18.10		3.88	1.94	7.61		1.09	0.27	4.29
	SMX		58.60	50.66	66.12		58.06	51.34	64.50		54.37	47.47	61.10		52.17	44.89	59.36
	TAZ		4.46	2.12	9.12		3.69	1.84	7.23		1.46	0.47	4.46		0.54	0.08	3.82
	TET		56.69	48.75	64.30		58.06	51.34	64.50		52.43	45.55	59.22		44.02	36.96	51.34
	TIG		/	/	/		/	/	/		/	/	/		0.00	/	/
	TMP		50.32	42.47	58.15		52.53	45.83	59.15		48.54	41.73	55.41		50.00	42.76	57.24

		2015				2016				2017				2018			
Veal calves	AMP	196	57.65	50.57	64.44	174	55.75	48.22	63.02	185	58.38	51.08	65.33	190	69.47	62.5	75.7
	AZI		4.08	2.04	7.99		4.02	1.92	8.25		4.86	2.53	9.14		4.21	2.11	8.24
	CHL		27.55	21.70	34.28		25.29	19.33	32.34		28.65	22.54	35.64		31.58	25.3	38.6
	CIP		23.98	18.47	30.51		19.54	14.26	26.18		21.62	16.23	28.20		23.68	18.1	30.3
	COL		2.04	0.76	5.35		1.72	0.55	5.26		1.08	0.27	4.27		2.11	0.79	5.52
	FOT		3.06	1.37	6.69		4.02	1.92	8.25		4.32	2.16	8.46		8.95	5.61	14
	GEN		6.63	3.87	11.14		4.02	1.92	8.25		5.41	2.92	9.81		5.26	2.84	9.55
	MER		0.00	/	/		0.00	/	/		0.00	/	/		0.00	/	/
	NAL		21.94	16.65	28.33		18.39	13.27	24.92		11.35	7.49	16.84		17.37	12.6	23.5
	SMX		56.12	49.04	62.97		59.77	52.25	66.86		57.84	50.54	64.81		70	63.1	76.1
	TAZ		3.57	1.70	7.35		2.87	1.19	6.77		4.86	2.53	9.14		7.37	4.39	12.1
	TET		61.22	54.16	67.84		70.69	63.43	77.03		65.95	58.76	72.47		75.79	69.1	81.4
	TIG		0.00	/	/		0.00	/	/		0.00	/	/		0.53	0.07	3.7
	TMP		40.31	33.61	47.38		40.80	33.68	48.33		54.59	47.31	61.69		64.21	57.1	70.8
Beef cattle	AMP	180	14.44	9.99	20.44	176	15.34	10.70	21.52	120	20.00	13.70	28.24	151	21.85	15.9	29.2
	AZI		1.11	0.27	4.39		1.14	0.28	4.49		5.00	2.24	10.80		1.32	0.33	5.22
	CHL		10.56	6.81	16.02		10.23	6.51	15.71		15.00	9.60	22.68		12.58	8.13	19
	CIP		4.44	2.22	8.69		5.68	3.07	10.29		12.50	7.62	19.83		7.28	4.05	12.8
	COL		0.00	/	/		0.57	0.08	3.99		0.00	/	/		0	/	/
	FOT		3.33	1.49	7.27		1.14	0.28	4.49		6.67	3.34	12.88		6.62	3.57	12
	GEN		5.00	2.61	9.38		3.98	1.89	8.16		5.83	2.78	11.85		8.61	5.03	14.3
	MER		0.00	/	/		0.00	/	/		0.83	0.11	5.82		0	/	/
	NAL		3.89	1.85	7.98		5.11	2.66	9.59		9.17	5.11	15.92		3.97	1.78	8.63
	SMX		25.00	19.17	31.91		26.70	20.64	33.79		22.50	15.82	30.96		41.06	33.4	49.2
	TAZ		2.78	1.15	6.55		0.57	0.08	3.99		5.00	2.24	10.80		8.61	5.03	14.3
	TET		15.56	10.92	21.67		19.32	14.10	25.89		19.17	13.01	27.33		20.53	14.8	27.8
	TIG		0.00	/	/		0.00	/	/		0.83	0.11	5.82		0	/	/
	TMP		10.56	6.81	16.02		11.93	7.88	17.67		17.50	11.63	25.49		23.84	17.7	31.4
Chickens	AMP	152	75.66	68.12	81.89	167	83.83	77.37	88.72	159	76.73	69.45	82.71	151	84.77	78	89.7
	AZI		3.29	1.36	7.73		1.20	0.30	4.72		2.52	0.94	6.58		1.32	0.33	5.22
	CHL		19.08	13.54	26.20		25.15	19.10	32.36		24.53	18.41	31.89		29.14	22.4	37
	CIP		63.82	55.79	71.14		57.49	49.79	64.83		57.86	49.97	65.37		60.26	52.2	67.8
	COL		0.00	/	/		0.00	/	/		0.00	/	/		0.66	0.09	4.65
	FOT		4.61	2.19	9.42		10.18	6.39	15.83		20.13	14.55	27.16		19.21	13.6	26.4
	GEN		7.24	4.03	12.67		3.59	1.61	7.83		7.55	4.31	12.89		10.60	6.56	16.7
	MER		0.00	/	/		0.00	/	/		0.00	/	/		0	/	/
	NAL		61.84	53.79	69.29		48.50	40.94	56.14		52.83	44.98	60.54		54.30	46.23	62.16
	SMX		68.42	60.52	75.38		68.86	61.37	75.48		62.26	54.40	69.53		76.82	69.34	82.93
	TAZ		4.61	2.19	9.42		8.98	5.46	14.43		17.61	12.40	24.41		15.23	10.29	21.97
	TET		48.68	40.75	56.69		51.50	43.86	59.06		50.31	42.51	58.10		56.29	48.19	64.07
	TIG		0.00	/	/		0.00	/	/		3.14	0.28	4.46		1.32	0.33	5.22
	TMP		53.95	45.90	61.79		56.89	49.19	64.26		50.31	42.51	58.10		61.59	53.51	69.08
Pigs	AMP	186	35.48	28.88	42.69	173	46.82	39.44	54.35	177	51.98	44.56	59.31	185	49.19	42	56.4
	AZI		0.00	/	/		1.16	0.29	4.56		2.26	0.84	5.92		2.7	1.12	6.38
	CHL		17.74	12.86	23.97		24.28	18.41	31.29		25.99	20.01	33.02		25.41	19.6	32.2
	CIP		2.15	0.80	5.64		5.78	3.12	10.47		9.60	6.03	14.97		7.57	4.51	12.4
	COL		0.00	/	/		1.16	0.29	4.56		1.13	0.28	4.46		0	/	/
	FOT		1.08	0.27	4.25		2.31	0.86	6.05		10.73	6.92	16.28		11.35	7.49	16.8
	GEN		1.61	0.52	4.93		0.58	0.08	4.06		1.13	0.28	4.46		3.24	1.45	7.08
	MER		0.00	/	/		0.00	/	/		0.00	/	/		0	/	/
	NAL		1.61	0.52	4.93		1.73	0.55	5.29		4.52	2.26	8.83		3.78	1.8	7.77
	SMX		43.01	36.02	50.29		50.29	42.81	57.75		48.02	40.69	55.44		58.38	51.1	65.3
	TAZ		1.08	0.27	4.25		2.31	0.86	6.05		10.17	6.47	15.63		10.27	6.62	15.6
	TET		40.32	33.45	47.59		46.82	39.44	54.35		47.46	40.14	54.89		47.57	40.4	54.8
	TIG		0.00	/	/		0.00	/	/		1.13	0.28	4.46		1.08	0.27	4.27
	TMP		38.17	31.41	45.42		45.09	37.76	52.63		45.76	38.50	53.21		53.51	46.2	60.6

Table 2 summarizes the data obtained from 2011 to 2018. It shows the number of sample analysed, the prevalence of resistant isolates (means, low and high confidences intervals (%)) for each animal category and each tested antimicrobial substance.

- AMP: ampicillin; AZI: Azithromycin; CHL: chloramphenicol; CIP: ciprofloxacin; COL: colistin; FOT: cefotaxime; GEN: gentamicin; MER: Meropenem NAL: nalidixic acid; SMX: suphamethoxazole; TAZ: ceftazidime; TET: tetracycline; TIG: Tigecyclin TMP: trimethoprim.
- N= number of tested samples.
- % resistance: mean prevalence of resistant isolates and confidence intervals (L.C.I.: lower confidence interval and U.C.I.: upper confidence interval) in per cent (%).

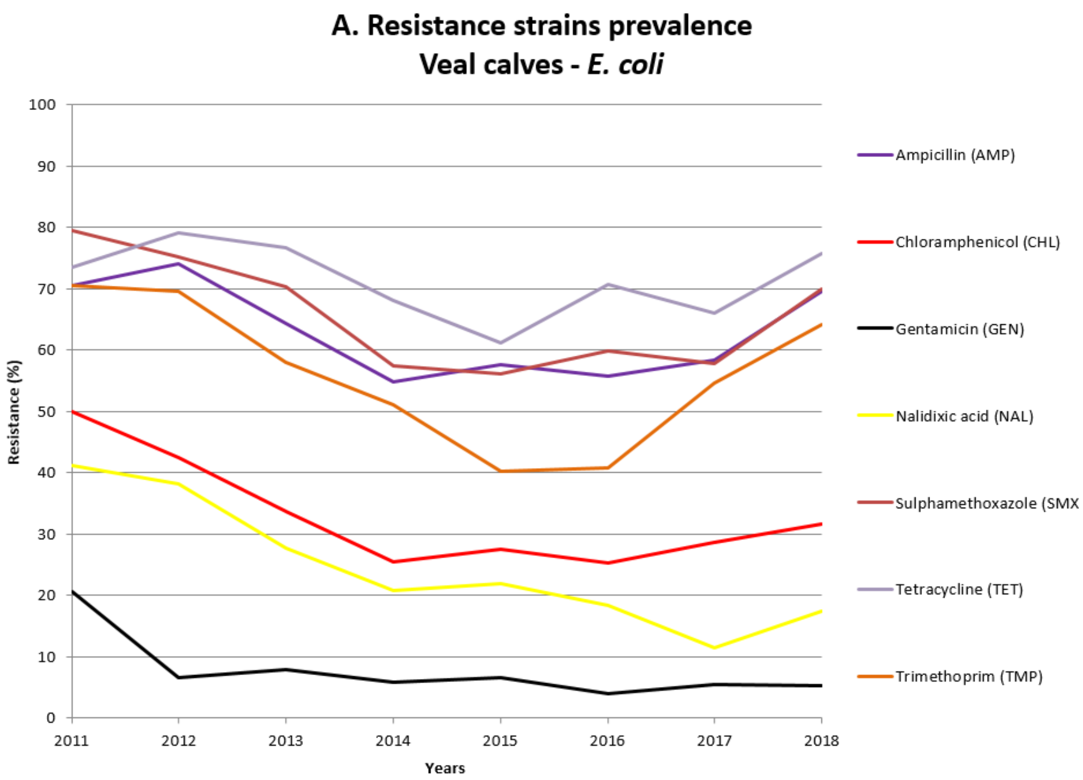
B.Trend analysis

Detailed outputs of the multiple comparisons corrections are presented in Annex 2. In this report the adjective 'high' was used in case of a prevalence of resistant strains higher than 50%. However, the significance of a given level of resistance will depend on the particular antimicrobial and its importance in human and veterinary medicine. The non-linear mixed multivariate model was chosen for the results interpretation as it gave an akaike information criterion (AIC) slightly lower than the AIC of the logistic model.

a) Veal Calves

As shown in **figure 1a**, an increase in resistance prevalence in 2018 was observed for all antimicrobial excepted for AZI (-0.65%), GEN (-0.15%) and MERO (0%) compared to 2017. High prevalence of resistance (>50%) was observed for the eight consecutive years for TET, SMX, AMP. For TMP, prevalence of resistance was > 40% for the eight consecutive years and >50% in 2011, 2012, 2013, 2017, 2018. The highest increases in veal calves were noted in 2018 compared to 2017 for AMP (+11.09%), SMX (+12.16%), TET (+9.84%) and TMP (+9.62%). Regarding TMP, increases in resistance have been observed for the last two years: +13.8% between 2016 and 2017 and + 9.62% between 2017 and 2018.

Figure 1a shows that resistance globally decreased for NAL in 2018 compared to 2011 (41.18% in 2011 versus 17.37% in 2018) even if an increase was observed between 2017 and 2018 (+6.02%). **Figure 1b**, shows the critical antimicrobials, based on the World Health Organisation antimicrobials classification (World Health Organisation, 2017). For these substances, resistance globally decreased for CIP (41.18% in 2011 versus 23.68% in 2018) and remained low for the others (<10%) during the whole study period.



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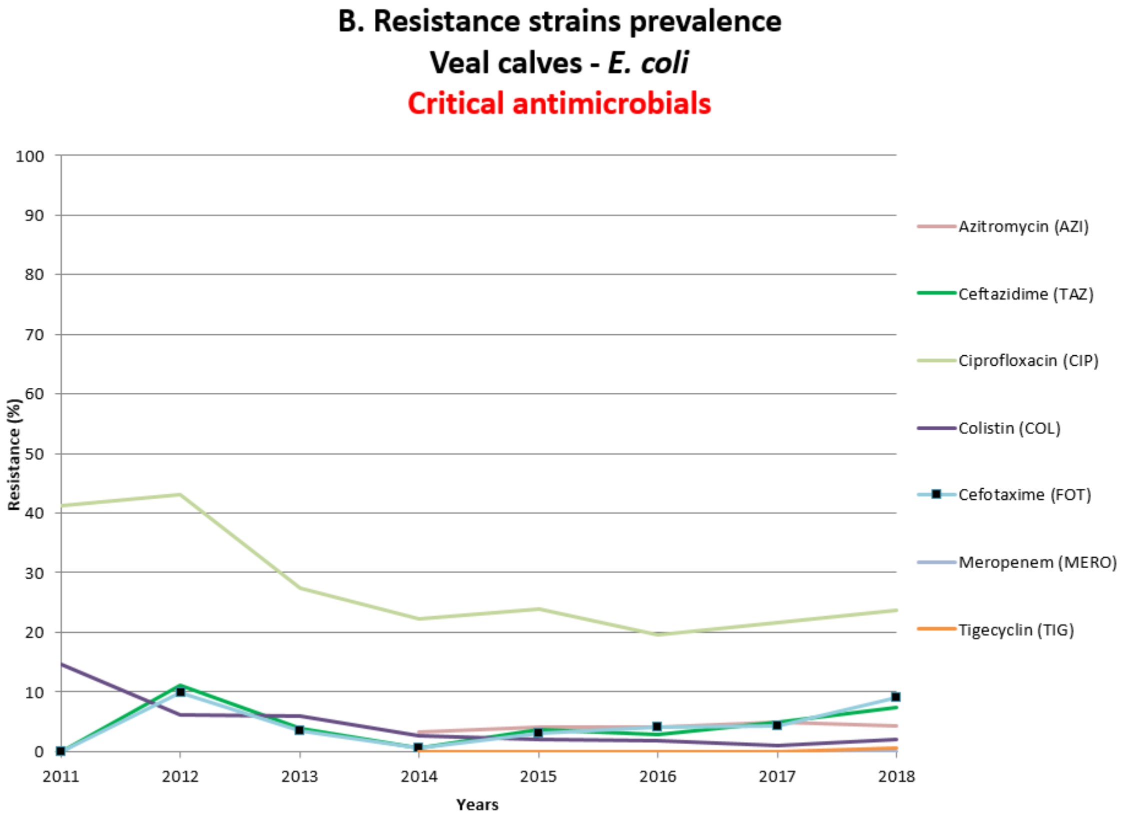

Figures 1a and 1b. Resistance strains prevalence: veal calves

Figures 1a and 1b describe the antimicrobial susceptibility trends of faecal *E. coli* retrieved from veal calves in Belgium (2011-2018).

Based on the results of the linear multivariate model (GEE), the probability to be resistant decreases significantly over time (2011-2018) for all tested substances except for AMP, TET, TMP,TAZ,FOT, GEN (**figure 2**).

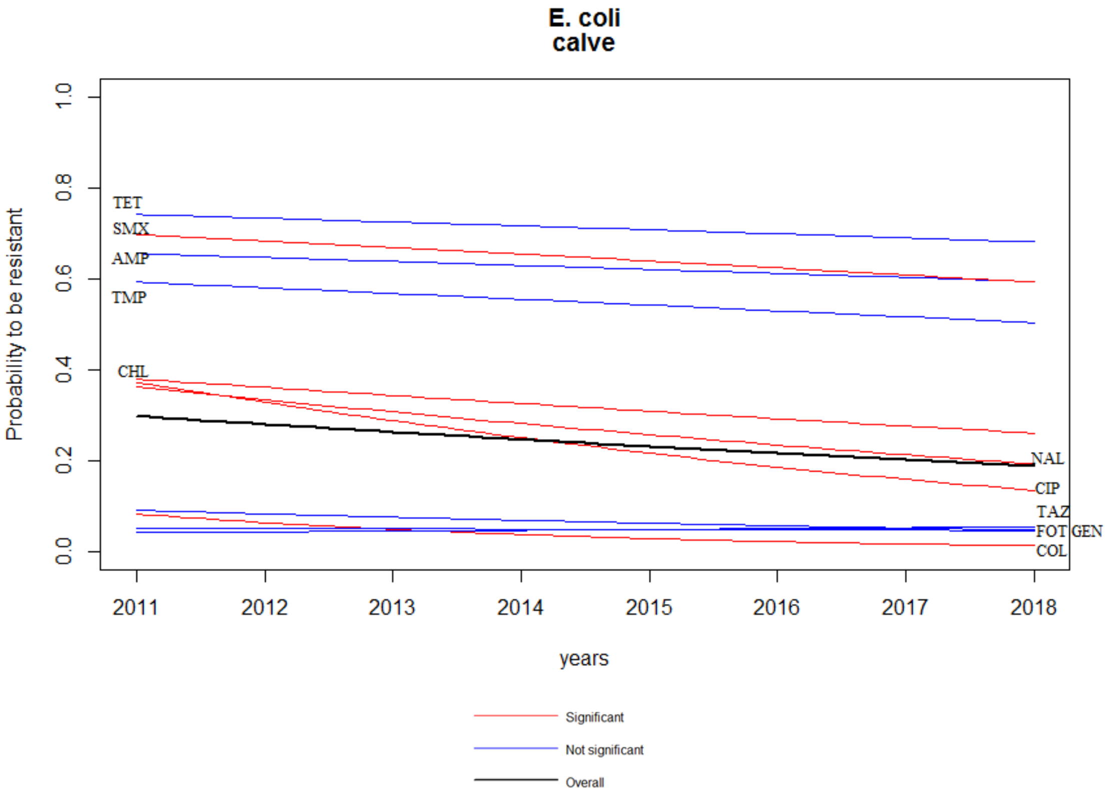

Figure 2 displays results of the linear multivariate model (GEE) of faecal E. coli retrieved from veal calves in Belgium (2011-2018).

The detailed odds ratios obtained from the non-linear mixed multivariate model are shown in **table 3** and the log odds of the logistic regression were plotted (see annexes). Based on the non-linear mixed multivariate model we notice a significant decrease in resistance (odds ratio (OR)<1)) compared to the year before for all substances from 2012 to 2014. However, since 2012 OR have been increasing in all substances compared to the year before. By consequence, decrease in resistance was no more significant for any substance since 2017 and some substances have shown significant and persisting increases in resistance (OR>1) compared to the year before: since 2016 for AMP and FOT, since 2017 for SMX, TAZ and TMP, since 2018 for CHL and TET

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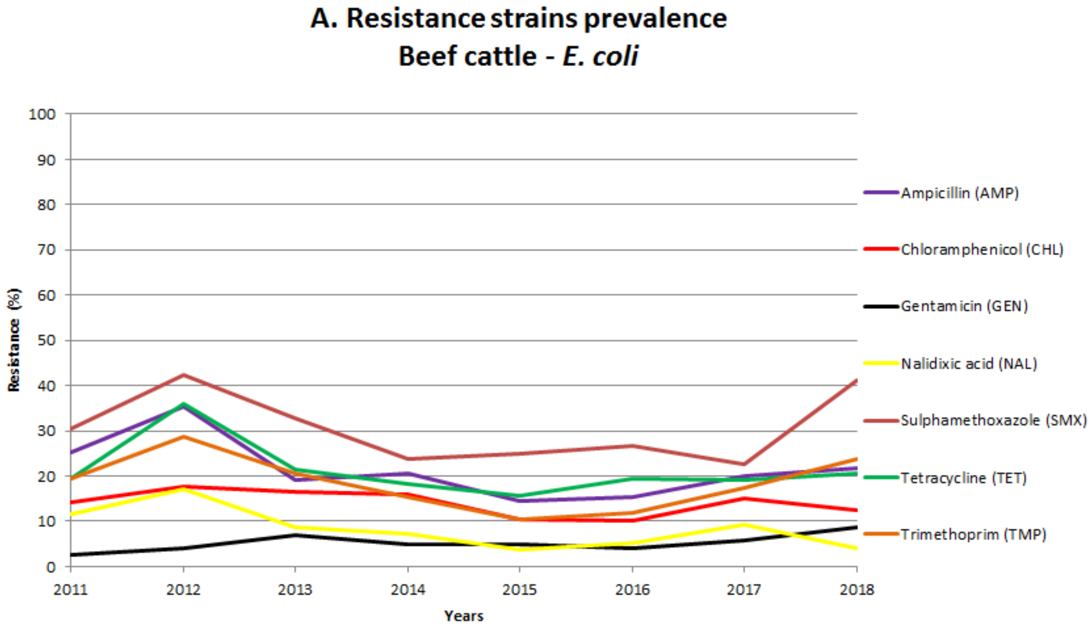
Table 3. Odds ratio of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016			OR7: year 2018 vs 2017		
	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit
AMP	**0.62**	0.52	0.73	**0.76**	0.71	0.81	**0.90**	0.87	0.94	**1.05**	0.96	1.13	**1.19**	1.04	1.33	**1.33**	1.13	1.53	**1.47**	1.21	1.74
CHL	**0.65**	0.54	0.76	**0.70**	0.59	0.80	**0.79**	0.71	0.86	**0.88**	0.83	0.93	**0.99**	0.92	1.06	**1.11**	0.99	1.23	**1.25**	1.04	1.45
CIP	**0.64**	0.52	0.75	**0.69**	0.58	0.79	**0.76**	0.69	0.84	**0.85**	0.79	0.90	**0.94**	0.87	1.01	**1.05**	0.92	1.17	**1.16**	0.95	1.37
COL	**0.58**	0.36	0.80	**0.62**	0.41	0.82	**0.68**	0.54	0.82	**0.74**	0.63	0.85	**0.82**	0.64	1.00	**0.90**	0.59	1.21	**0.99**	0.50	1.47
FOT	**0.55**	0.40	0.70	**0.61**	0.43	0.79	**0.76**	0.61	0.91	**0.96**	0.85	1.06	**1.20**	1.04	1.35	**1.50**	1.16	1.83	**1.87**	1.25	2.50
GEN	**0.70**	0.48	0.92	**0.75**	0.55	0.94	**0.81**	0.67	0.94	**0.87**	0.78	0.96	**0.94**	0.82	1.07	**1.02**	0.80	1.25	**1.11**	0.75	1.46
NAL	**0.67**	0.54	0.80	**0.70**	0.59	0.82	**0.75**	0.67	0.83	**0.80**	0.75	0.85	**0.86**	0.79	0.93	**0.91**	0.79	1.04	**0.98**	0.79	1.17
SMX	**0.58**	0.49	0.68	**0.64**	0.53	0.74	**0.74**	0.66	0.83	**0.87**	0.81	0.93	**1.01**	0.95	1.08	**1.18**	1.05	1.31	**1.01**	1.15	1.61
TAZ	**0.55**	0.40	0.70	**0.60**	0.43	0.78	**0.74**	0.60	0.88	**0.92**	0.81	1.02	**1.13**	0.98	1.28	**1.39**	1.07	1.71	1.71	1.13	2.29
TET	0.68	0.55	0.81	0.74	0.61	0.86	0.82	0.73	0.91	0.91	0.85	0.97	1.01	0.94	1.09	1.13	1.00	1.26	1.26	1.04	1.48
TMP	0.55	0.47	0.63	0.60	0.51	0.69	0.73	0.65	0.80	0.88	0.83	0.93	1.06	1.00	1.13	1.29	1.15	1.42	1.55	1.30	1.80

OR: odds ratio
Dark green: significant decrease; light green: non-significant decrease; orange: non-significant increase

b) Beef cattle

Globally, lower prevalence of resistance were observed in *E. coli* from beef cattle compared to veal calves. However, the highest resistance prevalence were noted against the same substances than for veal calves: AMP, SMX, TET and TMP (**figure 3a**). SMX has presented the highest prevalence of resistance for the whole study period and the prevalence still increased in 2018 (+18.56% compared to 2017). Important increase was also observed in 2018 for TMP (+6.34%). Between 2016 and 2017, prevalence increased by >5% for CIP, FOT (critical antimicrobials) and TMP. However, in 2018 resistance decreased by 5.22% for CIP and by 0.05% for FOT (**figure 3b**).



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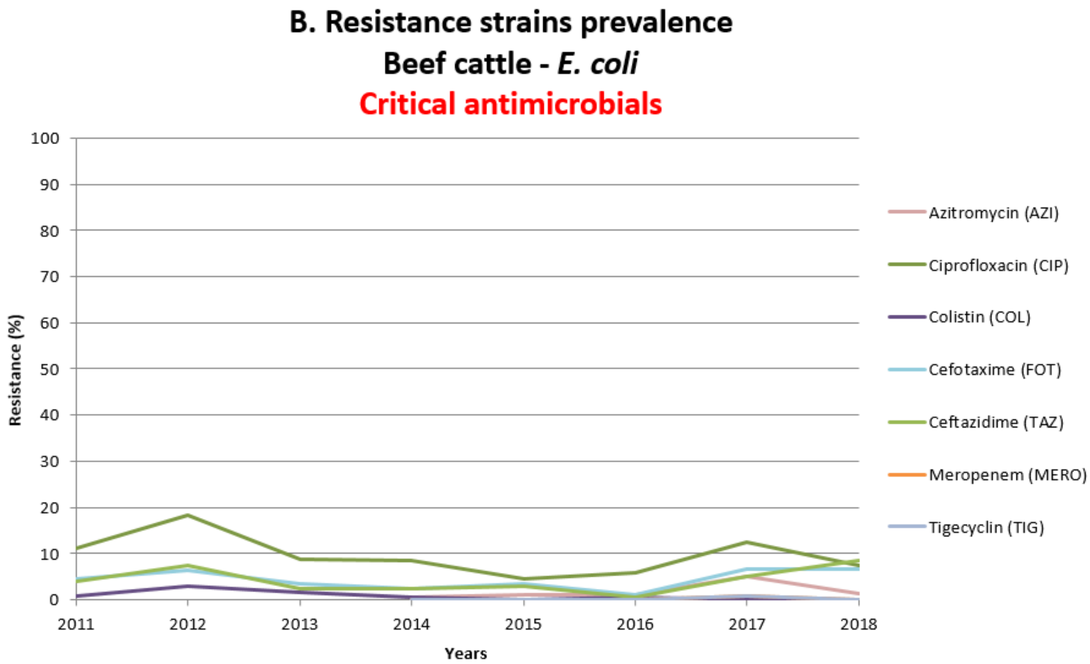

Figure 3a and 3b. Resistance strains prevalence: beef cattle

These figures 3a and 3b describe the antimicrobial susceptibility trends of faecal E. coli retrieved from beef cattle in Belgium (2011-2018).

Based on the results of the linear multivariate model (GEE), the probability to be resistant decreases significantly over time for AMP, NAL, CIP and COL (**figure 4**).

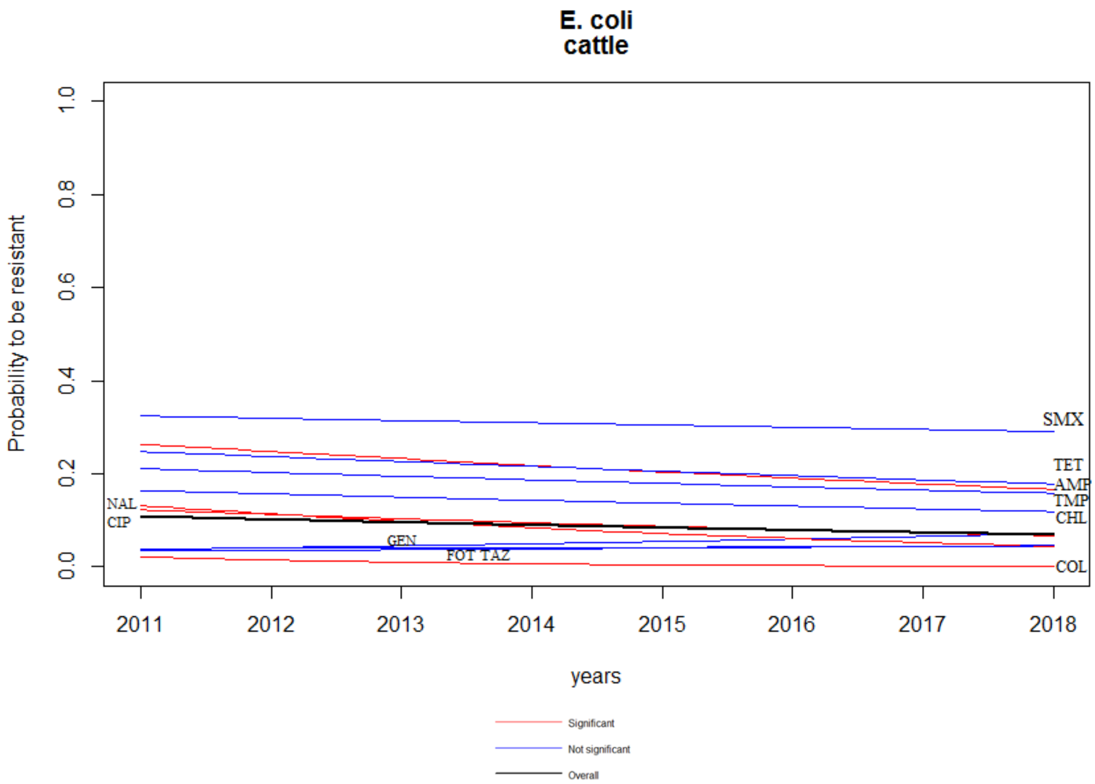

Figure 4 displays results of the linear multivariate model (GEE) of faecal E. coli retrieved from beef cattle in Belgium (2011-2018).

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The detailed OR obtained from the non-linear mixed multivariate model are shown in **table 4** and the log odds of the logistic regression were plotted (see annexes). Based on the non-linear mixed multivariate model we notice a constant significant decrease in resistance (OR<1) compared to the year before for FOT (years 2012-2013), AMP, TAZ, TMP (years 2012-2014) and for CIP, NAL, TET (years 2012-2015). However, OR progressively increased over time for all substances except for COL. These increases became significant for SMX, TAZ since 2016 and for TMP since 2018. COL is the only substance that has shown a continuous decrease in resistance since 2012 (OR<1 since 2014) and this decrease has been significant from 2015. However, prevalence for COL was already low.

Table 4. Odds ratio of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016			OR7: year 2018 vs 2017		
	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit
AMP	0.74	0.62	0.86	0.82	0.75	0.88	0.89	0.85	0.94	0.97	0.90	1.05	1.05	0.92	1.17	1.12	0.94	1.31	1.20	0.96	1.44
CHL	0.92	0.73	1.11	0.93	0.79	1.07	0.94	0.85	1.02	0.94	0.88	1.01	0.95	0.85	1.05	0.96	0.81	1.11	0.97	0.75	1.19
CIP	0.74	0.58	0.90	0.79	0.65	0.92	0.84	0.75	0.93	0.91	0.83	0.98	0.97	0.85	1.09	1.04	0.84	1.24	1.12	0.82	1.42
COL	1.85	0.00	4.49	1.03	0.39	1.68	0.65	0.29	1.01	0.40	0.00	0.81	0.25	0.00	0.65	0.16	0.00	0.50	0.10	0.00	0.38
FOT	0.70	0.49	0.90	0.77	0.57	0.97	0.88	0.74	1.02	1.01	0.90	1.12	1.16	0.98	1.34	1.33	1.00	1.66	1.52	0.98	2.06
GEN	1.04	0.67	1.41	1.06	0.79	1.32	1.07	0.90	1.25	1.09	0.97	1.21	1.11	0.95	1.26	1.12	0.87	1.37	1.14	0.77	1.51
NAL	0.74	0.57	0.91	0.77	0.63	0.90	0.80	0.71	0.89	0.84	0.76	0.92	0.87	0.75	1.00	0.91	0.71	1.12	0.95	0.66	1.25
SMX	0.77	0.66	0.89	0.83	0.73	0.92	0.90	0.83	0.96	0.98	0.93	1.03	1.25	1.05	1.44	1.15	1.02	1.28	1.25	1.04	1.45
TAZ	0.63	0.46	0.80	0.71	0.52	0.89	0.85	0.72	0.99	1.03	0.92	1.14	1.25	1.05	1.44	1.50	1.13	1.88	1.81	1.16	2.46
TET	0.86	0.71	1.00	0.88	0.77	0.99	0.91	0.84	0.98	0.94	0.88	0.99	0.97	0.89	1.05	1.00	0.87	1.14	1.03	0.84	1.23
TMP	0.72	0.60	0.83	0.78	0.67	0.88	0.86	0.79	0.93	0.95	0.89	1.01	1.05	0.96	1.14	1.16	1.00	1.32	1.28	1.04	1.53

c) Broiler Chickens

A high prevalence of resistance was observed for broiler chickens with values ≥ 50% for the eight consecutive years for AMP, CIP (critical antimicrobial) and SMX and with values ≥ 50% for seven years for NAL and TMP (**figures 5a and 5b**). Prevalence of resistance increased by 9.95% and by 8.63% for FOT and for TAZ respectively from 2016 to 2017 but slightly decreased in 2018 (-0.92% and -2.38% respectively). However, increases by 14.56% for SMX, by 8.04% for AMP and by 11.28% for TMP were observed in 2018. A slight but constant increase of resistance since 2016 is pointed out for CHL (+10% from 2016 to 2018).

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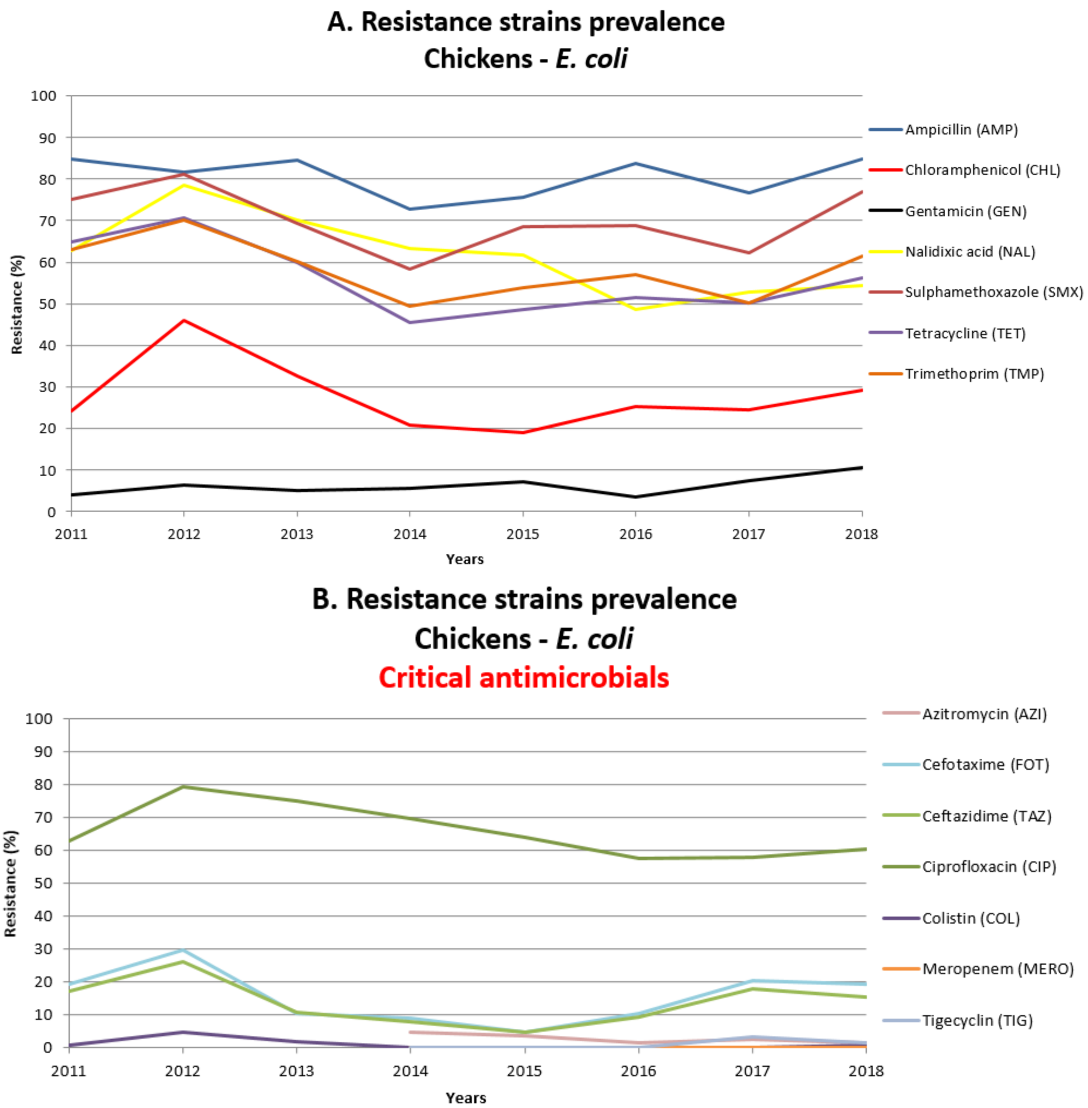


Figure 5a and 5b. Resistance strains prevalence: chickens.

Figures 5a and 5b describe the antimicrobial susceptibility trends of faecal E. coli retrieved from chickens in Belgium (2011-2018).

Based on the results of the linear multivariate model (GEE), the probability to be resistant significantly decreases over time for all tested substances except for GEN, AMP and CHL (**figure 6**). For GEN, resistance statistically increases but prevalence remains slow (<10%).

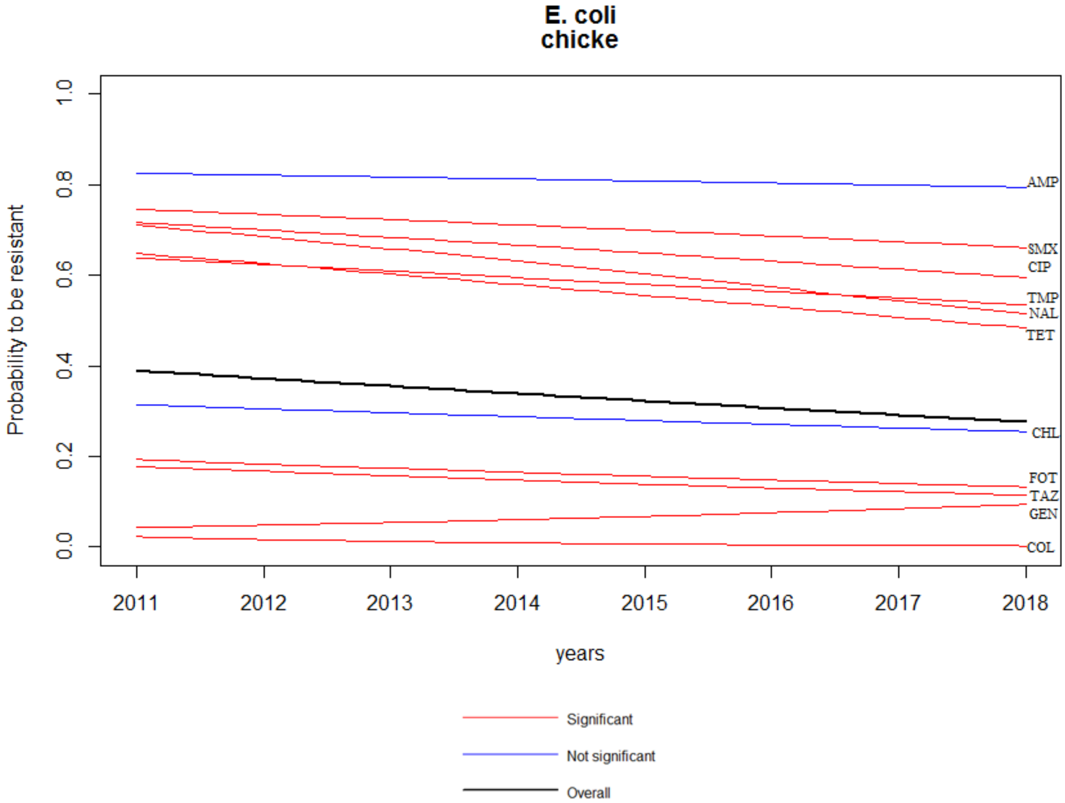


Figure 6 displays results of the linear multivariate model (GEE) of faecal E. coli retrieved from chickens in Belgium (2011-2018).

The detailed odds ratios obtained from the non-linear mixed multivariate model were shown in **table 5** and the log odds of the logistic regression were plotted (see annexes). In the last years, it can be observed a decreasing trend in resistance (OR<1) when comparing to the year before for CHL (significant in 2015), for CIP (significant since 2015), in COL (significant since 2014), for NAL (significant since 2012). It should be mentioned that AMP (since 2015), SMX (since 2016) present odds ratio >1, however not significant. Compared to the year before, significant increases in resistance were highlighted in FOT (since 2017), TAZ (since 2017) and GEN (2015).

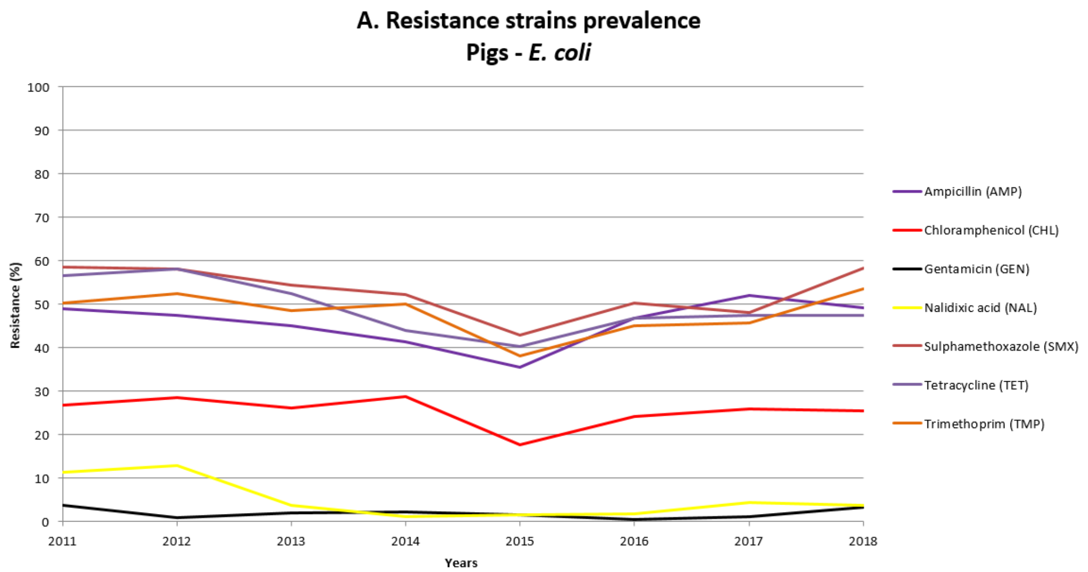
Table 5: Odds ratio of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016			OR7: year 2018 vs 2017		
	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit
AMP	0.82	0.70	0.94	0.88	0.81	0.96	0.95	0.90	0.99	1.01	0.94	1.07	1.07	0.96	1.19	1.13	0.97	1.30	1.20	0.98	1.42
CHL	0.97	0.84	1.10	0.96	0.88	1.05	0.96	0.90	1.01	0.95	0.90	0.99	0.95	0.87	1.01	0.93	0.83	1.04	0.93	0.78	1.08
CIP	1.10	0.95	1.25	1.03	0.94	1.12	0.97	0.92	1.02	0.91	0.87	0.95	0.86	0.80	0.92	0.81	0.72	0.89	0.76	0.65	0.87
COL	1.35	0.29	2.40	0.95	0.59	1.32	0.71	0.46	0.95	0.52	0.21	0.84	0.39	0.03	0.75	0.29	0.00	0.65	0.21	0.00	0.56
FOT	0.68	0.59	0.77	0.75	0.67	0.83	0.85	0.79	0.91	0.96	0.91	1.02	1.09	1.00	1.19	1.24	1.07	1.41	1.42	1.14	1.68
GEN	1.02	0.75	1.29	1.05	0.86	1.24	1.08	0.95	1.20	1.10	1.01	1.19	1.13	0.99	1.28	1.16	0.93	1.40	1.20	0.86	1.53
NAL	0.99	0.86	1.12	0.95	0.87	1.03	0.91	0.86	0.96	0.88	0.84	0.92	0.85	0.79	0.90	0.82	0.73	0.90	0.79	0.67	0.90
SMX	0.79	0.69	0.89	0.84	0.76	0.91	0.90	0.84	0.95	0.96	0.92	1.00	1.03	0.95	1.10	1.10	0.98	1.23	1.18	1.00	1.37
TAZ	0.70	0.60	0.80	0.76	0.67	0.85	0.85	0.79	0.91	0.95	0.89	1.00	1.06	0.96	1.15	1.18	1.01	1.35	1.31	1.04	1.58
TET	0.78	0.69	0.87	0.82	0.75	0.89	0.86	0.82	0.91	0.92	0.88	0.96	0.97	0.91	1.03	1.03	0.92	1.13	1.08	0.93	1.24
TMP	0.84	0.74	0.95	0.88	0.80	0.95	0.91	0.86	0.96	0.95	0.91	0.99	0.99	0.92	1.05	1.03	0.92	1.13	1.07	0.91	1.22

OR: odds ratio
Dark green: significant decrease; light green: non-significant decrease; orange: non-significant increase; red: significant increase

d) Pigs

The prevalences of resistance for AMP and, TMP were above 40% during seven years (2011-2014/2016-2018) and during the eight consecutive years for TET and SMX (**figure 7a**). AMP was in 2017 for the first time the antimicrobial with the highest prevalence in pigs (4th from 2011 to 2015) but resistance slightly decreased in 2018 (-2.79%) and AMP felt to the 3th highest prevalence rank. Prevalences for FOT and for TAZ increased by +8.42% and by +7.86% respectively between 2016 and 2017 but remained stable in 2018. SMX increased by 10.36% in 2018. For COL and GEN, prevalences of these two substances were very low (<4%) from 2011 to 2018(**figures 7a, 7b)**.



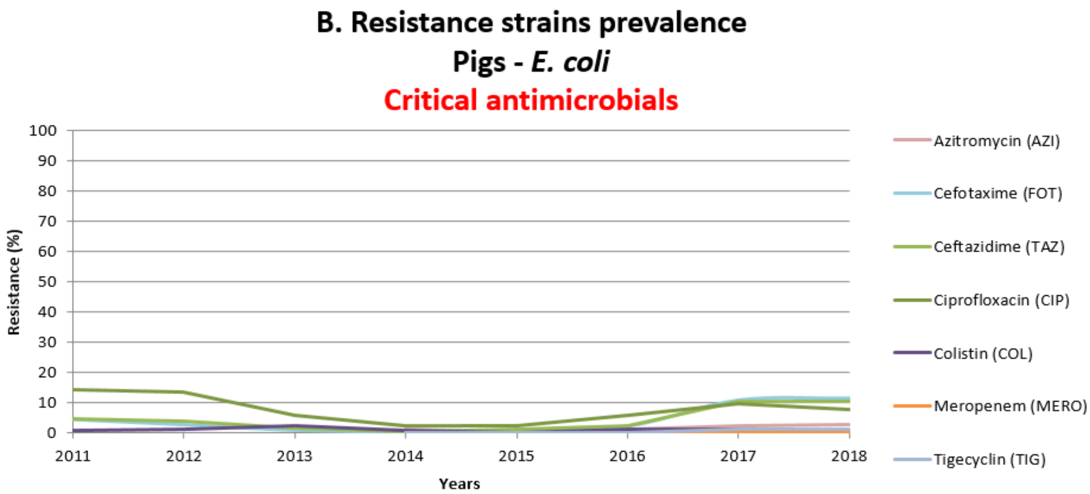


Figure 7a and 7b. Resistance strains prevalence: pigs

Figures 7a and 7b describe the antimicrobial susceptibility trends of faecal *E. coli* retrieved from pigs in, Belgium (2011-2018).

Based on the results of the linear multivariate model (GEE) (**figure8**), the probability to be resistant decreased significantly over time for TET and NAL and significantly increased for FOT and TAZ.

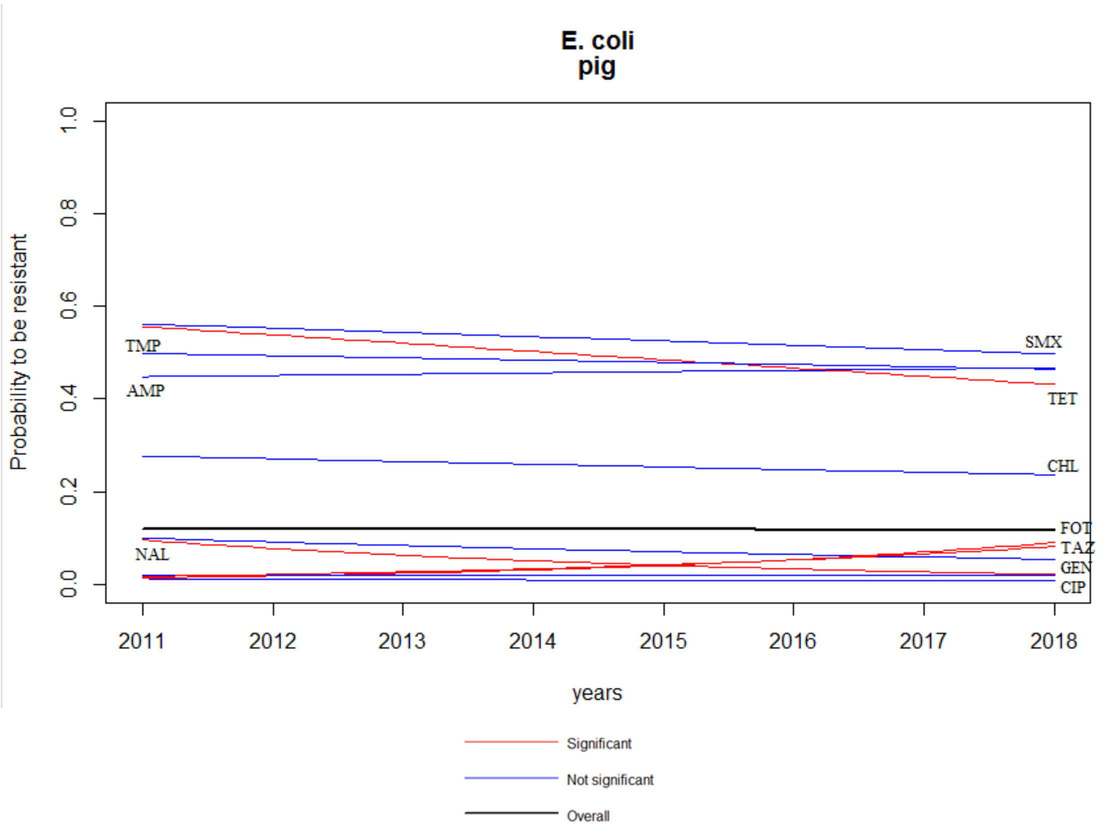


Figure 8 displays results of the linear multivariate model (GEE) of faecal *E. coli* retrieved from pigs in Belgium (2011-2018).

The detailed odds ratios obtained from the non-linear mixed multivariate model are shown in **table 6** and the log odds of the logistic regression were plotted (see annexes). Based on the non-linear multivariate model we notice that, except for COL, there is a constant increase in resistance. The model shows significant increases in resistance for AMP (since 2016), CIP (since 2017), FOT (since 2015), SMX (2016 vs 2015 and 2018 vs 2017) and TAZ (since 2015).

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Table 6: Odds ratio of the non-linear mixed multivariate model by antimicrobial substance and by years

Substance	OR1: year 2012 vs 2011			OR2: year 2013 vs 2012			OR3: year 2014 vs 2013			OR4: year 2015 vs 2014			OR5: year 2016 vs 2015			OR6: year 2017 vs 2016			OR7: year 2018 vs 2017		
	Estimate	Lower limit	Upper limit	**Estimate**	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit	Estimate	Lower limit	Upper limit
AMP	**0.84**	0.73	0.96	**0.91**	0.83	0.98	**0.97**	0.93	1.01	**1.03**	0.98	1.07	**1.09**	1.01	1.17	**1.15**	1.03	1.28	**1.21**	1.04	1.38
CHL	**0.90**	0.76	1.05	**0.92**	0.82	1.03	**0.95**	0.88	1.02	**0.97**	0.92	1.02	**1.00**	0.93	1.07	**1.02**	0.91	1.14	**1.05**	0.88	1.22
CIP	**0.56**	0.46	0.66	**0.62**	0.51	0.74	**0.75**	0.67	0.84	**0.91**	0.84	0.98	**1.10**	0.98	1.23	**1.34**	1.08	1.59	**1.61**	1.18	2.05
COL	**1.27**	0.16	2.38	**1.10**	0.49	1.70	**0.96**	0.63	1.30	**0.85**	0.60	1.10	**0.74**	0.40	1.09	**0.65**	0.20	1.11	**0.57**	0.02	1.13
FOT	**0.61**	0.45	0.77	**0.71**	0.50	0.92	**0.93**	0.76	1.10	**1.22**	1.09	1.35	**1.59**	1.38	1.80	**2.08**	1.60	2.56	**2.72**	1.79	3.66
GEN	**0.69**	0.40	0.98	**0.76**	0.49	1.03	**0.86**	0.67	1.06	**0.98**	0.84	1.13	**1.12**	0.87	1.36	**1.27**	0.82	1.71	**1.44**	0.72	2.17
NAL	**0.51**	0.41	0.61	**0.55**	0.44	0.67	**0.67**	0.59	0.76	**0.81**	0.73	0.89	**0.99**	0.83	1.14	**1.20**	0.90	1.50	**1.45**	0.94	1.96
SMX	**0.79**	0.69	0.90	**0.84**	0.75	0.93	**0.90**	0.84	0.96	**0.96**	0.92	1.01	**1.50**	1.30	1.70	**1.10**	0.99	1.21	**1.18**	1.01	1.34
TAZ	**0.61**	0.45	0.77	**0.71**	0.51	0.91	**0.91**	0.75	1.07	**1.17**	1.05	1.29	**1.50**	1.30	1.70	**1.93**	1.49	2.36	**2.47**	1.64	3.30
TET	**0.79**	0.68	0.89	**0.83**	0.74	0.91	**0.88**	0.82	0.94	**0.93**	0.89	0.97	**0.99**	0.93	1.05	**1.05**	0.94	1.15	**1.11**	0.95	1.27
TMP	**0.85**	0.73	0.97	**0.89**	0.80	0.98	**0.94**	0.87	1.00	**0.98**	0.94	1.03	**1.03**	0.97	1.10	**1.08**	0.98	1.19	**1.14**	0.98	1.30

OR: odds ratio
Dark green: significant decrease; light green: non-significant decrease; orange: non-significant increase; red: significant increase

5. Multi-resistance

➢ Prevalence of multi-resistance

The proportion of multi-resistant strains (= strains resistant to at least three antimicrobial famillies) was very high for broiler chickens (>62%) and high for veal calves (>50%) during the eight consecutive years (**Table 7** and **Figure 9**). In pigs and veal calves multi-resistance continuously increased since 2016. In beef, multi-resistance increased since 2017 after four consecutive years of decrease (2013-2016). In chickens and in veal calves, multi-resistance increased by 10% and by 12.70% from 2017 to 2018 respectively.

Figure 10 displays the distribution of multi-resistance patterns per animal category (i.e, number of isolates resistant to 0, 1….9 of the antimicrobial classes tested).
13.16%, 49.01%, 6.85 %, 23.78%, of, respectively, meat calves, young bovine, chicken and pig isolates, were fully susceptible (=no resistance) in 2018 to all tested antimicrobials. Compared to 2017, the proportion of fully susceptible strains decreased by 13%, 23%, 4% and 3% in meat calves, young bovine, chicken and pig respectively.

Table 7: Proportion of multi-resistant strains (%) (+95% confidence interval)

	Veal calves	Beef cattle	Chickens	Pigs
2011	**70.59** (54.45-86.73)	**24.68** (17.79-31.56)	**77.86** (73.87-81.84)	**53.50** (45.62-61.39)
2012	**72.93** (66.39-79.46)	**32.57** (25.56-39.58)	**81.88** (77.63-86.12)	**53.92** (47.23-60.6)
2013	**66.83** (60.28-73.38)	**23.04 (**17.21-28.87)	**76.92** (71.48-82.36)	**48.54** (41.66-55.43)
2014	**56.38** (49.23-63.54)	**20.73** (14.46-27)	**62.03** (54.37-69.68)	**47.83** (40.54-55.11)
2015	**51.02** (43.96-58.08)	**16.67** (11.17-22.16)	**70.39** (63.05-77.73)	**36.56** (29.57-43.54**)**
2016	**58.05** (50.64-65.45)	**15.91** (10.45-21.37)	**68.86** (61.77-75.96)	**45.09** (37.60-52.57)
2017	**56.76** (49.55-63.96)	**22.50** (14.92-30.08)	**67.30** (59.92-74.67)	**48.02** (40.59-55.45)
2018	**69.47** (62.87-76.08)	**23.84** (16.97-30.72)	**77.48** (70.74-84.22)	**51.89** (44.62-59.16)

Table 7 shows the proportion (%) and 95% confidence interval of multi-resistance from faecal *E. coli* retrieved from veal calves, beef cattle, chickens and pigs in Belgium (2011-2018).

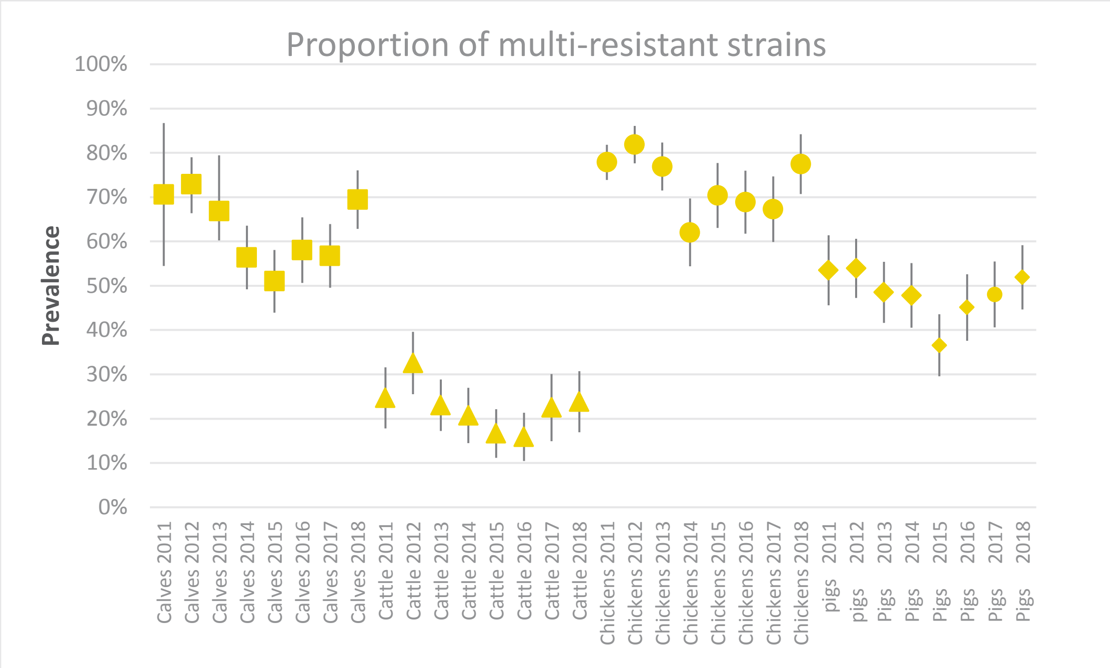
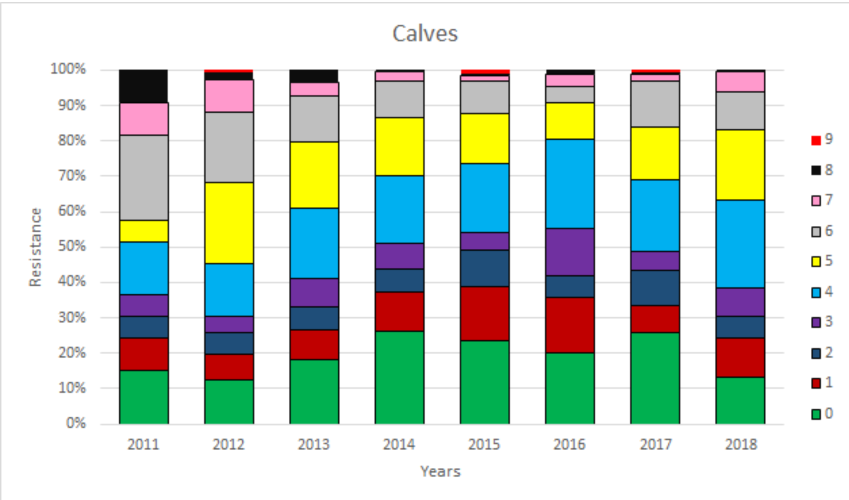


Figure 9. Proportion of multi-resistant strains (+95% Confidence intervals).

Figure 9 graphically represents multi-resistance prevalence, for veal calves, beef cattle, chickens and pigs and by years (same data displayed on table 6).



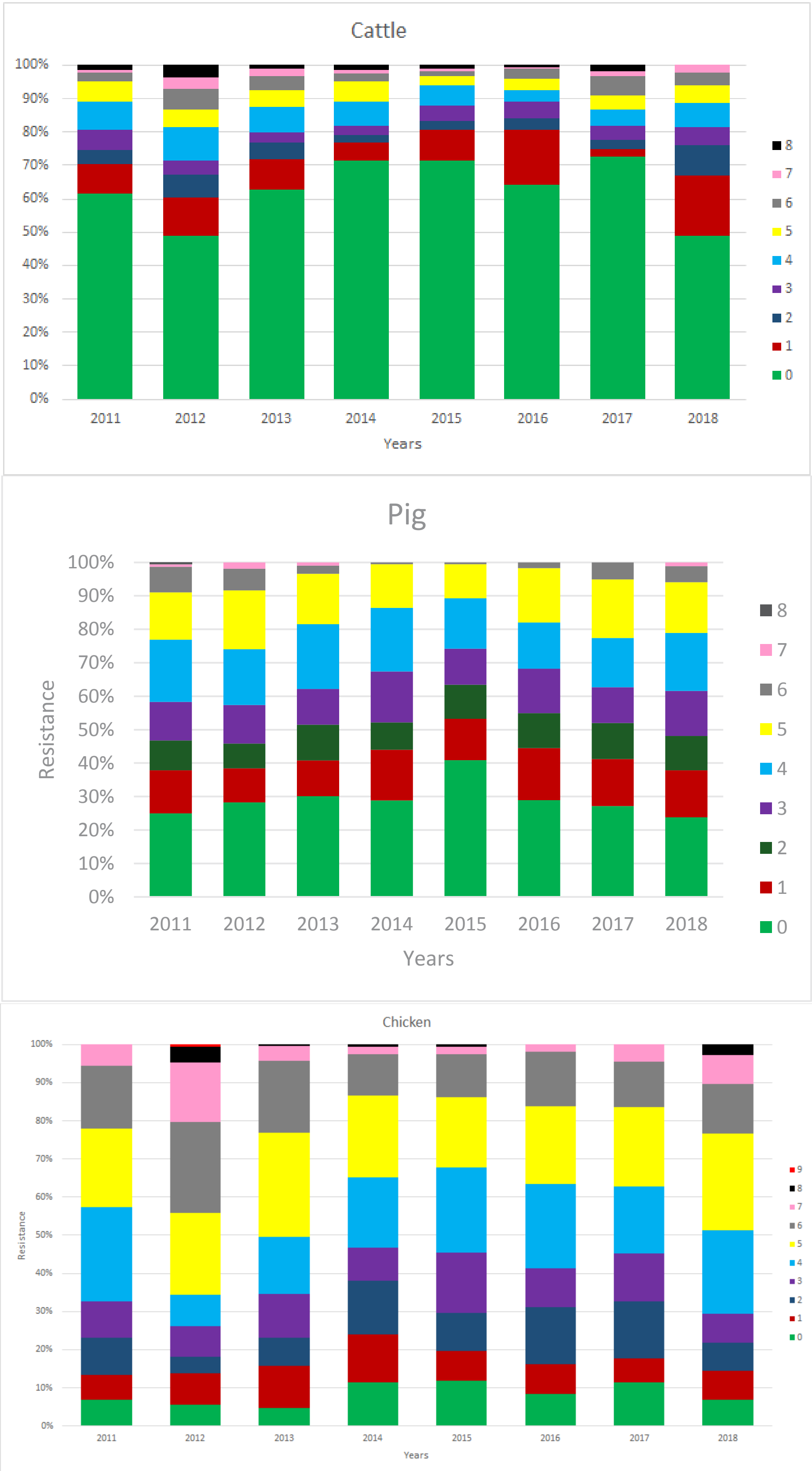


Figure 10. Distribution of multi-resistance patterns (%) per animal category and by years (2011-2018). 0= fully sensitive to 9= resistant to 9 different antimicrobials classes.

Table 8 and 9 present the OR (the ratio of the odds for a one-unit increase in the time) for multi-resistance obtained from the linear and non-linear models, respectively. In 2017, all species showed significant decreases in multi-resistance. In 2018, this decrease in multi-resistance was only significant in beet cattle and chickens but at the limit of the non-significant threshold.
In 2018 a significant increase in multi-resistance was confirmed in veal calves and in chickens.

Table 8. Ratio of the odds and confidence intervals for multi-resistance obtained from the linear model (2011-2018), by species category.

Species	OR	95%CI
Veal calves	0.952	0.902-1.004
Beef cattle	0.935	0.882-0.992
Chickens	0.935	0.894-0.979
Pigs	0.971	0.928-1.016

OR= odds ratio; 95%CI= 95% confidence intervals

Table 9: Ratio of the odds and confidence intervals regarding to probability to be multi-resistant (logistic regression, year by year)

Years compared	Veal calves	Beef cattle	Chickens	Pig
2012 vs 2011	1.263 (0.561-2.842)	1.405 (0.863-2.285)	1.350 (0.935-1.948)	1.039 (0.688-1.571)
2013 vs 2012	0.710 (0.455-1.108)	0.622 (0.394-0.980)	0.738 (0.487-1.118)	0.799 (0.545-1.171)
2014 vs 2013	0.643(0.426-0.970)	0.877(0.533-1.442)	0.492(0.316-0.765)	0.972(0.653-1.447)
2015 vs 2014	0.807 (0.540-1.206)	0.767 (0.446-1.318)	1.451 (0.904-2.330)	0.630 (0.416-0.955)
2016 vs 2015	1.326 (0.879-2.000)	0.947 (0.541-1.658)	0.931 (0.578-1.501)	1.422 (0.932 -2.170)
2017 vs 2016	0.949 (0.624-1.442)	1.533 (0.853-2.755)	0.931 (0.584-1.482)	1.124 (0.739-1.712)
2018 vs 2017	1.728 (1.131-2.641)	1.074 (0.609-1.895)	1.663 (1.004-2.755)	1.166 (0.772-1.762)

➢ Index of diversity: Weighted Entropy

The weighted entropy is a diversity index that reflects how many different patterns of resistance are present in a dataset, and simultaneously take into account how evenly the observed resistance patterns are distributed. The weighted entropy takes a value lower to 1 if the isolates are resistant to a higher number of antimicrobials. As shown in **table 10**, the value of the index decreased for all species comparing 2011 to 2018. A decrease of 24% was observed in 2018 in beef cattle. The index was globally lower for pigs compared with other species.

Table 10. Weighted Entropy by species category and by years.

Years	Veal calves	Beef cattle	Chickens	Pigs
2011	0.68	0.52	0.64	0.48
2012	0.71	0.63	0.79	0.48
2013	0.63	0.55	0.62	0.4
2014	0.54	0.59	0.59	0.32
2015	0.54	0.48	0.57	0.33
2016	0.50	0.41	0.58	0.36
2017	0.54	0.67	0.61	0.43
2018	0.56	0.43	0.61	0.46

Discussion

Prevalence

Eleven substances were tested phenotypically from 2011 to 2018 and 3 from 2014 to 2018 (AZI, MERO, TIG). The three antimicrobials tested from 2014 are not used in veterinary medicine and prevalence of resistance was very low (max 5%).

Discussion will focus now on the other eleven antimicrobials common for the eight years. The prevalence of resistance increased for 10/11 antimicrobial substances tested in 2018 compared to 2017 in veal calves, 6/11 in beef cattle, 9/11 in chickens and 6/11 in pigs.

The prevalence of resistance to the critical antimicrobials (CIP, FOT and TAZ) was stable in 2018 comparing to 2017 in every species.
The prevalence of resistance for SMX increased the most 2018 compared to 2017 in all animal categories (between 12% to 18%). AMP also increased in 2018 in veal calves and chickens (>8%).

There was globally a high level of resistance to AMP, SMX, TET and TMP in all animal species, but to a lesser extent in beef cattle. The common patterns of resistance to AMP, SMX, TMP and TET and combinations thereof often feature as a component of multi-resistance patterns, and are probably related to the presence of class 1 or class 2 integrons, which generally carry genes conferring resistance to these antimicrobials (Marchant et al., 2013). Although other risk factors have been described, antimicrobial use is recognized as the main selector for antimicrobial resistance and a correlation with resistance was pointed out in Belgium (Callens et al., 2017). In Belgium, antimicrobial sales data for use in animals are being collected on an annual basis since 2009 (BelVet-SAC, 2017). In 2017, a decrease of 25.9% in the sales of antimicrobials has been observed since 2011 and this reduction continued in 2018 (AMCRA, personal communication).

Trend analysis

GEE and NL mixed multivariate models present the lowest AIC but the other models used globally gave similar results.

Linear multivariate model (GEE)

In 2017, it was highlighted that there were more antimicrobials for which GEE results were non-significant compared to 2016. However, it could be assessed that the probability of *E. coli* to be antimicrobial resistant was overall significantly decreasing in Belgian production animals, with a lesser extend to pigs. In 2018 the situation was less clear except in chickens.
Considering the data of eight consecutive years (2011 to 2018), there were many antimicrobials for which the probability to be resistant non-significantly decreased: in veal calves (n=6 antimicrobials), in beef cattle (n=7), in chickens (n=2+1significant increase), and in pigs (n=7+2 significant increases). However, it should be nuanced for the substances that present resistance prevalence globally low (<10%) to very low (<5%) (ex: TAZ, FOT, COL in veal cattle, CIP, GEN in pigs).

Specific assessments

Veal calves

The levels of antimicrobial resistance were very high in veal calves for AMP, SMX and TET (more than 50% of isolates are resistant during the eight consecutive years). Major increases (>10%) in AMP and SMX were reported in 2018. TMP which prevalence of resistance was below 50%, since 2015 showed the most important increase observed in 2017 (+13.8%) and increased again in 2018 (+9.62%).
It cannot be affirmed by the non-linear analysis that the significant decreases observed for from 2012 to 2014-2015, depending on the substance, continued afterward. When comparing 2018 to 2017,, seven increases were significant by both NL mixed multivariate and logistic models

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(AMP,CHL,FOT,SMX,TAZ,TET,TMP). Attention should be given to resistance in calves because we have observed since 2017 OR>1 for 9/11 substances.
The GEE model highlighted the probability to be resistant significantly decrease for only five substances. However, FOT, COL, TAZ were non-significant but prevalence was low to extremely low.

Beef cattle
In beef cattle, resistance prevalence is globally lower than in other species. However, SMX shown the highest increase in resistance observed in 2018 (+18.56%). The GEE model highlighted the probability to be resistant significantly decrease for only four substances, including CIP and COL for which resistance was already low.
For SMX, TAZ, TMP a significant increase (OR>1) is highlighted by NL mixed multivariate in 2018 compared to 2017 (also by logistic procedure for TAZ and SMX). We should pay attention to these substances for which prevalence increased.

Chickens
Chickens present a high level of resistance to certain substances (e.i. AMP, SMX, CIP are >50% resistance during the 8 years). COL prevalence of resistance is 0% since 2014 (0.66% in 2018 but the positive strain needs laboratory confirmation).

Based on the GEE, the probability to be resistant for substances with high levels of resistance statistically significantly decreased over time, except for AMP. GEN significantly increased but prevalence remains low.

An increasing trend was previously detected by NL models for CIP in 2012 but afterwards, a constant decrease of resistance has been observed, significant since the last years. Globally, whatever the NL model used, there is a significant decreasing trend in resistance in COL and NAL. However, a particular attention should be paid on FOT and SMX which showed significant (or limit to be significant for TAZ) increases in resistance) since 2017.
The high resistance to quinolones in chickens is especially worrisome because of a higher resistance percentage for ciprofloxacin compared to NAL, suggesting the presence of plasmid mediated quinolone resistance (Strahilevitz et al., 2009).

Pigs
The prevalence of resistance for TET and SMX was above 40% during the eight consecutive years. AMP prevalence constantly increased from 2015 to 2017 but slightly decreased in 2018. Based on the results of the GEE, the probability to be resistant significantly decreased over time only for TET and NAL and even significantly increased for FOT and TAZ. However, resistance remains low for these substances. A significant increase in resistance are observed at least since 2017 compared to the previous year by both NL models for AMP, CIP, FOT and TAZ and since 2018 for SMX.

Multi-resistance

The proportion of **multi-resistant strains** (= strains resistant to at least three antimicrobials) is very high for broiler chickens (>62%) and high for veal calves (>50%) during the eight consecutive years.
After four consecutive years of decrease, multi-resistance increased in beef cattle in 2017 and in 2018. The proportion of fully susceptible strains decreased by 13% and 23% in veal calves and beef cattle respectively. However, in beet cattle it is due to an increase of 15.4% of strain resistance to one class of antimicrobial. *In fine*, multi-resistance increased just by 1.3%.
In veal calves and chickens, the increase of multi-resistance (+12.7% and + 10.8% respectively) is explained by the higher resistance for 4 and 5 antimicrobials belonging to different families (+ 4% and 5% respectively).

In 2017, from the linear and non-linear models and for all species, significant decreases in multi-resistance were observed from 2011 onwards. However in 2018 only beef cattle and chickens showed a significant decreases in multi-resistance by the linear model. The NL model showed a significant increase in multi-resistance in veal calves and in chickens compared to 2017. Even if the proportion of fully sensitive strains decreased in 2018 in beef cattle, the multi-resistance did not significantly increased (22.5% in 2017 versus 23.84% in 2018).

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ANNEX

List of antimicrobials tested in this report and Epidemiological cut-off values (ECOFF)
Resistant strain if MIC value of the isolate > Cut-off

Symbol	Antimicrobial	Cut-off value (mg/ml)
AMP	Ampicillin	8
AZI	Azithromycin	16
CHL	Chloramphenicol	16
CIP	Ciprofloxacin	0,064
COL	Colistin	2
FOT	Cefotaxime	0,25
GEN	Gentamicin	2
MER	Meropenem	0.125
NAL	Nalidixic acid	16
SMX	Sulphonamide	64
TAZ	Ceftazidime	0,5
TET	Tetracycline	8
TGC	Tigecyclin	1
TMP	Trimethoprim	2

Outputs of the univariate logistic regression model (odds ratio) comparing 2011 to 2018

SPECIES= veal calves

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits	
year at substance=AMP	0.958	0.908	1.011
year at substance=CHL	0.918	0.868	0.971
year at substance=CIP	0.873	0.822	0.927
year at substance=COL	0.742	0.633	0.870
year at substance=FOT	1.046	0.926	1.180
year at substance=GEN	0.896	0.805	0.998
year at substance=NAL	0.813	0.763	0.868
year at substance=SMX	0.931	0.882	0.983
year at substance=TAZ	0.980	0.868	1.106
year at substance=TET	0.955	0.901	1.011
year at substance=TMP	0.945	0.896	0.995

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SPECIES= beef cattle

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits	
year at substance=AMP	0.912	0.859	0.969
year at substance=CHL	0.943	0.878	1.012
year at substance=CIP	0.896	0.822	0.977
year at substance=COL	0.685	0.498	0.941
year at substance=FOT	1.012	0.897	1.143
year at substance=GEN	1.097	0.983	1.224
year at substance=NAL	0.827	0.753	0.908
year at substance=SMX	0.975	0.924	1.028
year at substance=TAZ	1.043	0.921	1.180
year at substance=TET	0.936	0.881	0.994
year at substance=TMP	0.945	0.887	1.007

SPECIES= chickens

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits0.981	
year at substance=AMP	0.970	0.922	1.020
year at substance=CHL	0.952	0.910	0.995
year at substance=CIP	0.924	0.886	0.964
year at substance=COL	0.742	0.588	0.936
year at substance=FOT	0.929	0.879	0.982
year at substance=GEN	1.100	1.014	1.195
year at substance=NAL	0.887	0.851	0.925
year at substance=SMX	0.945	0.905	0.987
year at substance=TAZ	0.914	0.862	0.969
year at substance=TET	0.905	0.869	0.943
year at substance=TMP	0.939	0.902	0.978

SPECIES= pigs

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits	
year at substance=AMP	1.012	0.967	1.059
year at substance=CHL	0.973	0.924	1.025
year at substance=CIP	0.899	0.823	0.981

Wald Confidence Interval for Odds Ratios			
Label	Estimate	95% Confidence Limits	
year at substance=COL	0.888	0.698	1.129
year at substance=FOT	1.353	1.195	1.533
year at substance=GEN	0.972	0.825	1.144
year at substance=NAL	0.767	0.685	0.860
year at substance=SMX	0.966	0.924	1.011
year at substance=TAZ	1.272	1.129	1.433
year at substance=TET	0.935	0.893	0.978
year at substance=TMP	0.984	0.940	1.029

Outputs of the univariate logistic regression model (odds ratio), by species and comparing two consecutive years

SPECIES= veal calves

Wald Confidence Interval for Odds Ratios AMP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.596	0.480	0.739
2013 vs 2012	0.685	0.585	0.801
2014 vs 2013	0.787	0.710	0.872
2015 vs 2014	0.904	0.850	0.962
2016 vs 2015	1.039	0.973	1.109
2017 vs 2016	1.194	1.070	1.332
2018 vs 2017	1.372	1.164	1.618

Wald Confidence Interval for Odds Ratios CHL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.632	0.514	0.777
2013 vs 2012	0.706	0.608	0.821
2014 vs 2013	0.790	0.717	0.871
2015 vs 2014	0.884	0.833	0.937
2016 vs 2015	0.988	0.924	1.057
2017 vs 2016	1.106	0.988	1.237
2018 vs 2017	1.237	1.047	1.460

Wald Confidence Interval for Odds Ratios CIP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.617	0.498	0.765
2013 vs 2012	0.686	0.587	0.801
2014 vs 2013	0.762	0.689	0.842
2015 vs 2014	0.846	0.796	0.900
2016 vs 2015	0.941	0.875	1.011
2017 vs 2016	1.045	0.926	1.179
2018 vs 2017	1.161	0.972	1.387

Wald Confidence Interval for Odds Ratios COL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.551	0.346	0.877
2013 vs 2012	0.610	0.440	0.846
2014 vs 2013	0.677	0.552	0.830
2015 vs 2014	0.750	0.649	0.867
2016 vs 2015	0.831	0.673	1.027
2017 vs 2016	0.922	0.658	1.290
2018 vs 2017	1.022	0.635	1.644

Wald Confidence Interval for Odds Ratios FOT			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.481	0.318	0.727
2013 vs 2012	0.604	0.447	0.815
2014 vs 2013	0.758	0.625	0.919
2015 vs 2014	0.952	0.851	1.065
2016 vs 2015	1.195	1.052	1.359
2017 vs 2016	1.501	1.203	1.872
2018 vs 2017	1.885	1.354	2.624

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.724	0.502	1.044
2013 vs 2012	0.774	0.595	1.007
2014 vs 2013	0.827	0.699	0.978
2015 vs 2014	0.883	0.795	0.981
2016 vs 2015	0.944	0.827	1.077
2017 vs 2016	1.008	0.811	1.254
2018 vs 2017	1.078	0.784	1.482

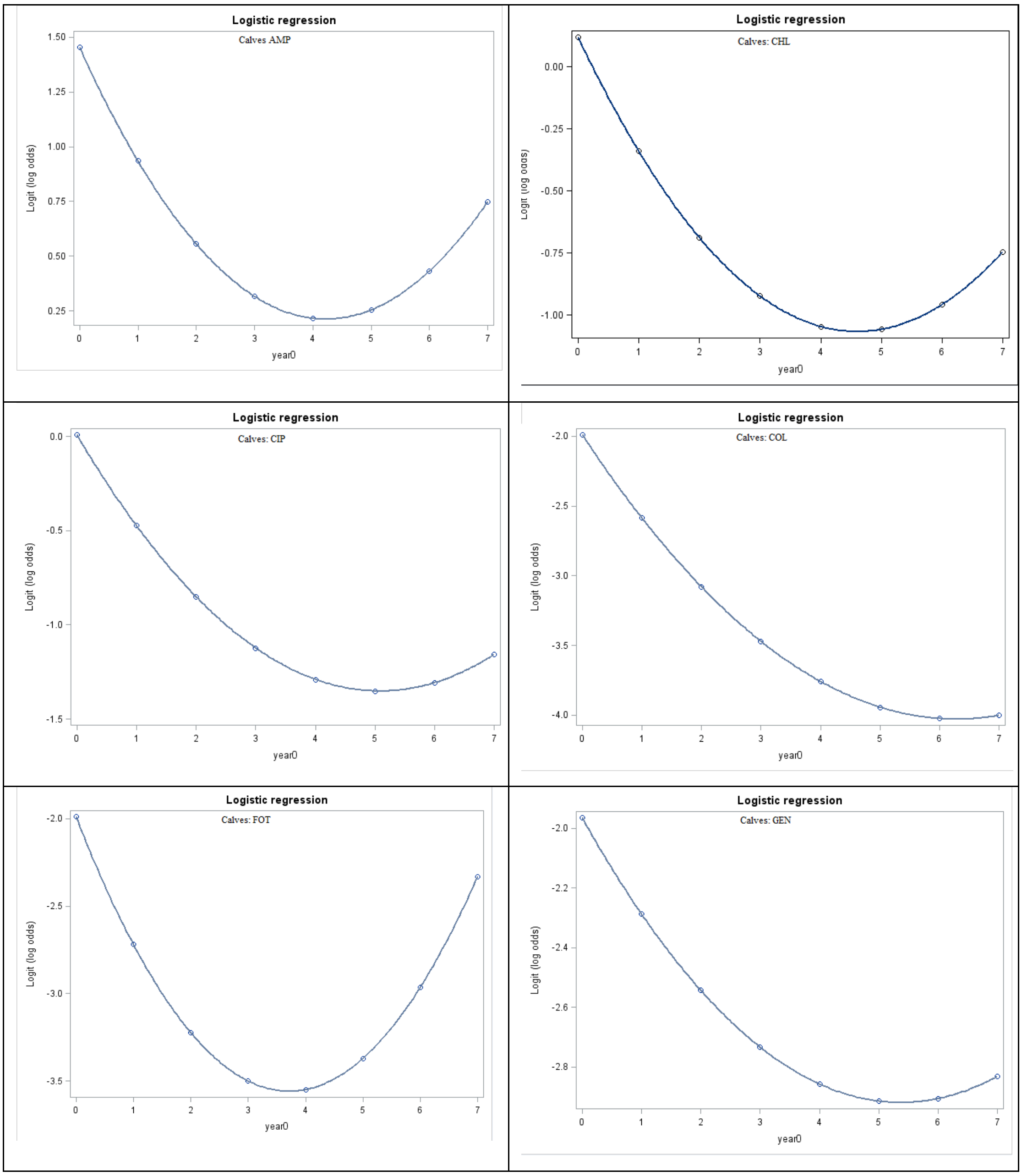
Wald Confidence Interval for Odds Ratios NAL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.662	0.529	0.828
2013 vs 2012	0.706	0.601	0.829
2014 vs 2013	0.753	0.679	0.834
2015 vs 2014	0.803	0.753	0.856
2016 vs 2015	0.857	0.790	0.929
2017 vs 2016	0.914	0.799	1.044
2018 vs 2017	0.975	0.802	1.185

Wald Confidence Interval for Odds Ratios SMX			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.552	0.442	0.690
2013 vs 2012	0.643	0.546	0.757
2014 vs 2013	0.748	0.672	0.833
2015 vs 2014	0.871	0.816	0.928
2016 vs 2015	1.013	0.948	1.082
2017 vs 2016	1.179	1.055	1.317
2018 vs 2017	1.372	1.161	1.621

Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.483	0.322	0.725
2013 vs 2012	0.598	0.446	0.801
2014 vs 2013	0.739	0.613	0.890
2015 vs 2014	0.913	0.818	1.019
2016 vs 2015	1.128	0.989	1.288
2017 vs 2016	1.395	1.113	1.747
2018 vs 2017	1.724	1.233	2.409

Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.664	0.528	0.836
2013 vs 2012	0.738	0.624	0.874
2014 vs 2013	0.820	0.734	0.917
2015 vs 2014	0.912	0.853	0.975
2016 vs 2015	1.014	0.945	1.087
2017 vs 2016	1.126	1.003	1.266
2018 vs 2017	1.252	1.051	1.491

Wald Confidence Interval for Odds Ratios TMP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.469	0.379	0.580
2013 vs 2012	0.575	0.492	0.673
2014 vs 2013	0.706	0.637	0.782
2015 vs 2014	0.966	0.815	0.921
2016 vs 2015	1.063	0.998	1.133
2017 vs 2016	1.305	1.172	1.453
2018 vs 2017	1.602	1.363	1.883


Logistic regression
Calves AMP
Logit (log odds)
1.50
1.25
1.00
0.75
0.50
0.25
0 1 2 3 4 5 6 7
year0
Logistic regression
Calves: CHL
Logit (log odds)
0.00
-0.25
-0.50
-0.75
-1.00
0 1 2 3 4 5 6 7
year0
Logistic regression
Calves: CIP
Logit (log odds)
0.0
-0.5
-1.0
-1.5
0 1 2 3 4 5 6 7
year0
Logistic regression
Calves: COL
Logit (log odds)
-2.0
-2.5
-3.0
-3.5
-4.0
0 1 2 3 4 5 6 7
year0
Logistic regression
Calves: FOT
Logit (log odds)
-2.0
-2.5
-3.0
-3.5
0 1 2 3 4 5 6 7
year0
Logistic regression
Calves: GEN
Logit (log odds)
-2.0
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-2.4
-2.6
-2.8
0 1 2 3 4 5 6 7
year0

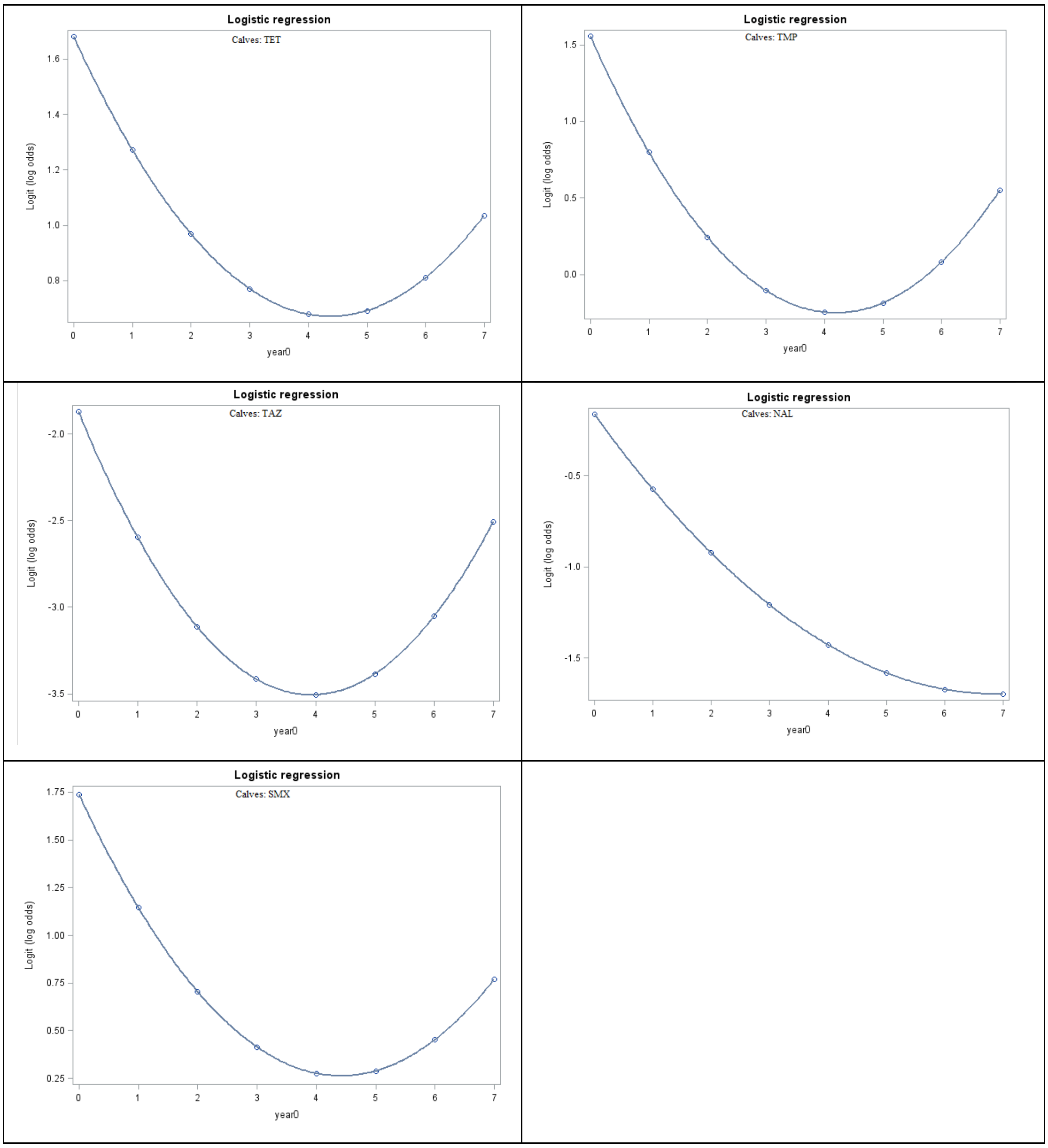


Figure 3. Logistic regression, by years.

Legend: year0: 0=2011; 1=2012; 2=2013; 3=2014; 4=2015 5=2016 6=2017; 7=2018.

Species= beef cattle

Wald Confidence Interval for Odds Ratios AMP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.730	0.611	0.872
2013 vs 2012	0.788	0.696	0.893
2014 vs 2013	0.851	0.787	0.921
2015 vs 2014	0.919	0.867	0.974
2016 vs 2015	0.993	0.912	1.080
2017 vs 2016	1.072	0.938	1.224
2018 vs 2017	1.157	0.961	1.394

Wald Confidence Interval for Odds Ratios CHL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.913	0.739	1.128
2013 vs 2012	0.923	0.795	1.071
2014 vs 2013	0.933	0.850	1.025
2015 vs 2014	0.944	0.880	1.013
2016 vs 2015	0.955	0.862	1.058
2017 vs 2016	0.966	0.823	1.132
2018 vs 2017	0.976	0.781	1.220

Wald Confidence Interval for Odds Ratios CIP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.732	0.574	0.934
2013 vs 2012	0.786	0.664	0.932
2014 vs 2013	0.845	0.760	0.939
2015 vs 2014	0.908	0.836	0.985

Wald Confidence Interval for Odds Ratios CIP			
Label	Estimate	95% Confidence Limits	
2016 vs 2015	0.975	0.863	1.102
2017 vs 2016	1.048	0.866	1.268
2018 vs 2017	1.126	0.863	1.468

Wald Confidence Interval for Odds Ratios COL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	1.033	0.448	2.384
2013 vs 2012	0.851	0.510	1.421
2014 vs 2013	0.701	0.482	1.020
2015 vs 2014	0.577	1.324	1.028
2016 vs 2015	0.475	0.190	1.188
2017 vs 2016	0.392	0.108	1.420
2018 vs 2017	0.322	0.061	1.716

Wald Confidence Interval for Odds Ratios FOT			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.658	0.462	0.938
2013 vs 2012	0.759	0.591	0.974
2014 vs 2013	0.875	0.748	1.023
2015 vs 2014	1.009	0.907	1.122
2016 vs 2015	1.163	0.999	1.355
2017 vs 2016	1.342	1.050	1.714
2018 vs 2017	1.547	1.091	2.193

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	1.057	0.743	1.504

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
2013 vs 2012	1.069	0.830	1.378
2014 vs 2013	1.082	0.918	1.274
2015 vs 2014	1.094	0.981	1.220
2016 vs 2015	1.107	0.963	1.273
2017 vs 2016	1.120	0.896	1.399
2018 vs 2017	1.133	0.823	1.560

Wald Confidence Interval for Odds Ratios NAL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.732	0.567	0.944
2013 vs 2012	0.766	0.643	0.913
2014 vs 2013	0.802	0.719	0.895
2015 vs 2014	0.840	0.765	0.922
2016 vs 2015	0.879	0.760	1.016
2017 vs 2016	0.920	0.738	1.147
2018 vs 2017	0.963	0.711	1.305

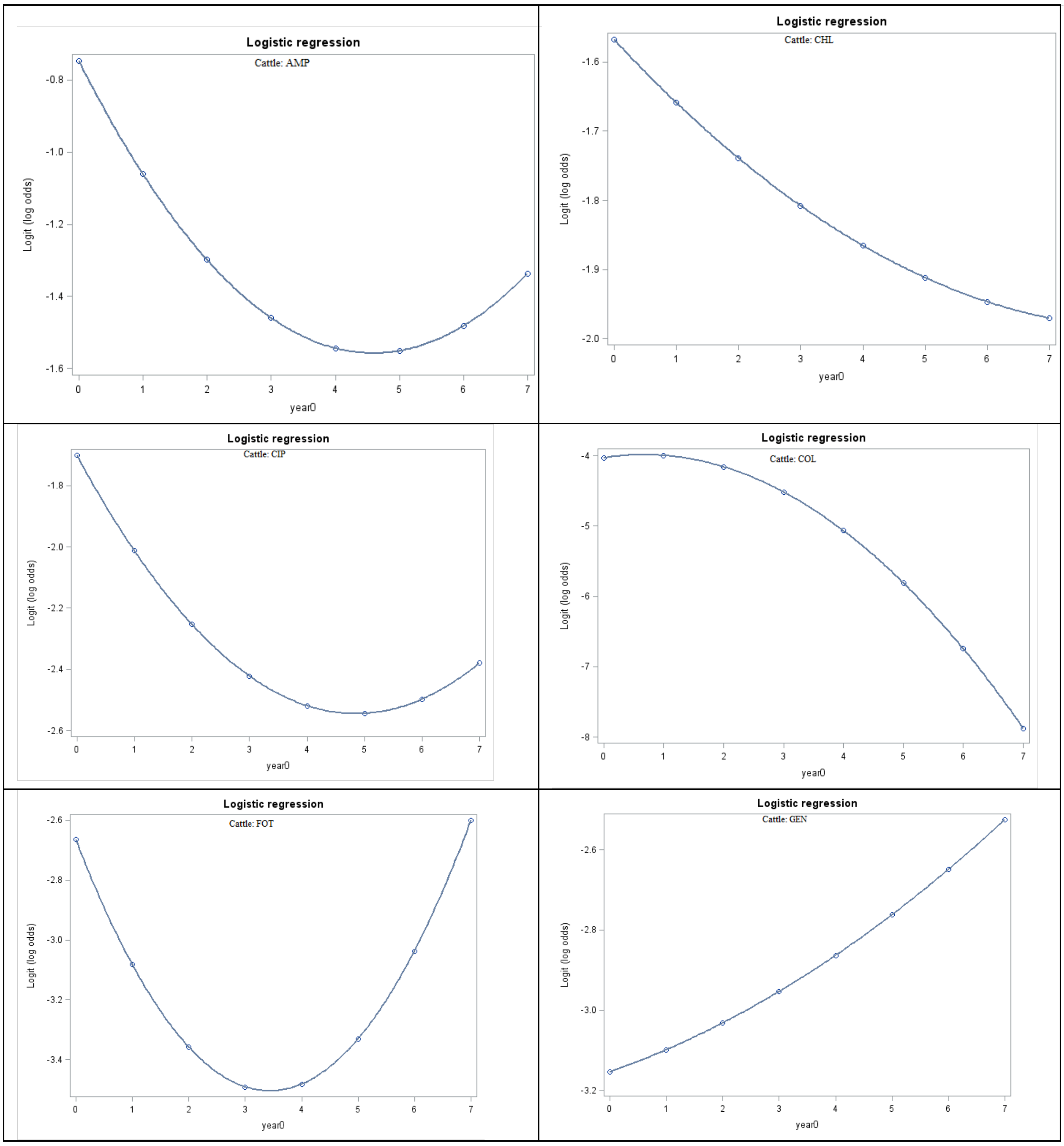
Wald Confidence Interval for Odds Ratios SMX			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.764	0.651	0.898
2013 vs 2012	0.829	0.740	0.930
2014 vs 2013	0.900	0.837	0.967
2015 vs 2014	0.976	0.927	1.028
2016 vs 2015	1.059	0.984	1.139
2017 vs 2016	1.149	1.024	1.289
2018 vs 2017	1.246	1.060	1.466

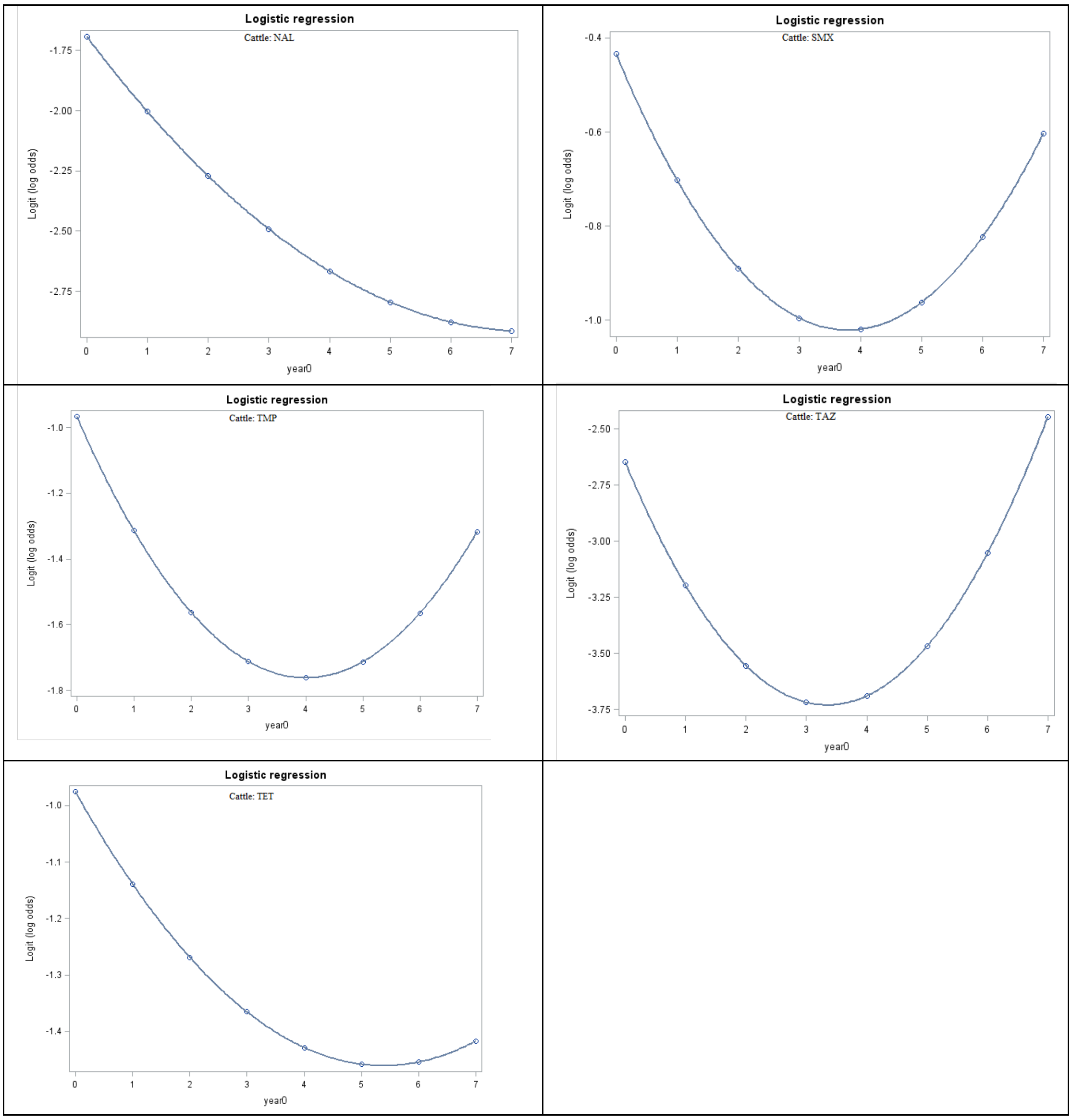
Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.576	0.400	0.831
2013 vs 2012	0.699	0.540	0.905
2014 vs 2013	0.848	0.723	0.996
2015 vs 2014	1.029	0.926	1.143
2016 vs 2015	1.248	1.073	1.452
2017 vs 2016	1.514	1.184	1.938
2018 vs 2017	1.837	1.290	2.616

Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.849	0.709	1.017
2013 vs 2012	0.878	0.774	0.997
2014 vs 2013	0.908	0.838	0.984
2015 vs 2014	0.939	0.885	0.996
2016 vs 2015	0.971	0.891	1.058
2017 vs 2016	1.004	0.878	1.148
2018 vs 2017	1.038	0.860	1.252

Wald Confidence Interval for Odds Ratios TMP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.706	0.596	0.851
2013 vs 2012	0.780	0.684	0.889
2014 vs 2013	0.861	0.793	0.935
2015 vs 2014	0.951	0.896	1.010
2016 vs 2015	1.050	0.963	1.146
2017 vs 2016	1.160	1.012	1.330

Wald Confidence Interval for Odds Ratios TMP			
Label	**Estimate**	**95% Confidence Limits**	
2018 vs 2017	1.281	1.057	1.553





Logistic regression, by years.

Legend: year0: 0=2011; 1=2012; 2=2013; 3=2014; 4=2015; 5=2016; 6=2017; 7=2018

Species=chickens

Wald Confidence Interval for Odds Ratios
AMP

Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.818	0.698	0.959
2013 vs 2012	0.870	0.780	0.970
2014 vs 2013	0.925	0.965	0.989
2015 vs 2014	0.983	0.932	1.038
2016 vs 2015	1.046	0.960	1.139

Wald Confidence Interval for Odds Ratios AMP			
Label	**Estimate**	**95% Confidence Limits**	
2017 vs 2016	1.112	0.973	1.270
2018 vs 2017	1.182	0.983	1.421

Wald Confidence Interval for Odds Ratios CHL			
Label	**Estimate**	**95% Confidence Limits**	
2012 vs 2011	0.972	0.849	1.111
2013 vs 2012	0.964	0.880	1.057
2014 vs 2013	0.957	0.905	1.012
2015 vs 2014	0.950	0.906	0.995
2016 vs 2015	0.942	0.874	1.016
2017 vs 2016	0.935	0.832	1.051
2018 vs 2017	0.928	0.790	1.090

Wald Confidence Interval for Odds Ratios CIP			
Label	**Estimate**	**95% Confidence Limits**	
2012 vs 2011	1.095	0.961	1.248
2013 vs 2012	1.030	0.942	1.127
2014 vs 2013	0.969	0.918	1.023
2015 vs 2014	0.912	0.874	0.952
2016 vs 2015	0.858	0.801	0.919
2017 vs 2016	0.807	0.725	0.898
2018 vs 2017	0.760	0.654	0.882

Wald Confidence Interval for Odds Ratios COL			
Label	**Estimate**	**95% Confidence Limits**	
2012 vs 2011	1.132	0.627	2.043

Wald Confidence Interval for Odds Ratios COL			
Label	Estimate	95% Confidence Limits	
2013 vs 2012	0.918	0.642	1.315
2014 vs 2013	0.745	0.560	0.993
2015 vs 2014	0.605	0.383	0.956
2016 vs 2015	0.491	0.240	1.003
2017 vs 2016	0.398	0.148	1.075
2018 vs 2017	0.323	0.090	1.160

Wald Confidence Interval for Odds Ratios FOT			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.661	0.561	0.778
2013 vs 2012	0.750	0.671	0.838
2014 vs 2013	0.851	0.796	0.909
2015 vs 2014	0.965	0.915	1.018
2016 vs 2015	1.095	1.002	1.196
2017 vs 2016	1.242	1.082	1.426
2018 vs 2017	1.409	1.163	1.707

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	1.009	0.776	1.313
2013 vs 2012	1.040	0.866	1.248
2014 vs 2013	1.072	0.958	1.198
2015 vs 2014	1.104	1.018	1.197
2016 vs 2015	1.137	1.005	1.288
2017 vs 2016	1.172	0.961	1.429
2018 vs 2017	1.207	0.913	1.596

Wald Confidence Interval for Odds Ratios NAL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.986	0.867	1.122
2013 vs 2012	0.950	0.869	1.037
2014 vs 2013	0.914	0.866	0.965
2015 vs 2014	0.880	0.844	0.918
2016 vs 2015	0.848	0.792	0.907
2017 vs 2016	0.816	0.734	0.907
2018 vs 2017	0.786	0.678	0.910

Wald Confidence Interval for Odds Ratios SMX			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.780	0.680	0.895
2013 vs 2012	0.836	0.760	0.819
2014 vs 2013	0.896	0.845	0.949
2015 vs 2014	0.959	0.916	1.005
2016 vs 2015	1.028	0.956	1.106
2017 vs 2016	1.101	0.983	1.233
2018 vs 2017	1.180	1.008	1.381

Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.684	0.577	0.812
2013 vs 2012	0.763	0.679	0.856
2014 vs 2013	0.850	0.793	0.911
2015 vs 2014	0.948	0.895	1.003
2016 vs 2015	1.056	0.961	1.160
2017 vs 2016	1.177	1.017	1.393

Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
2018 vs 2017	1.312	1.071	1.608

Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.775	0.683	0.879
2013 vs 2012	0.819	0.751	0.893
2014 vs 2013	0.867	0.822	0.914
2015 vs 2014	0.917	0.879	0.956
2016 vs 2015	0.970	0.907	1.036
2017 vs 2016	1.026	0.925	1.138
2018 vs 2017	1.085	0.939	1.253

Wald Confidence Interval for Odds Ratios TMP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.844	0.744	0.957
2013 vs 2012	0.877	0.805	0.956
2014 vs 2013	0.912	0.865	0.961
2015 vs 2014	0.948	0.909	0.989
2016 vs 2015	0.985	0.921	1.053
2017 vs 2016	1.024	0.923	1.136
2018 vs 2017	1.064	0.921	1.230

Species=pigs

Wald Confidence Interval for Odds Ratios AMP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.840	0.726	0.973
2013 vs 2012	0.893	0.805	0.991

Wald Confidence Interval for Odds Ratios AMP			
Label	Estimate	95% Confidence Limits	
2014 vs 2013	0.949	0.889	1.014
2015 vs 2014	1.009	0.964	1.055
2016 vs 2015	1.072	1.007	1.141
2017 vs 2016	1.139	1.031	1.259
2018 vs 2017	1.211	1.050	1.396

Wald Confidence Interval for Odds Ratios CHL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.903	0.766	1.065
2013 vs 2012	0.926	0.824	1.041
2014 vs 2013	0.949	0.881	1.022
2015 vs 2014	0.973	0.924	1.024
2016 vs 2015	0.997	0.928	1.071
2017 vs 2016	1.022	0.911	1.146
2018 vs 2017	1.048	0.890	1.232

Wald Confidence Interval for Odds Ratios CIP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.515	0.398	0.667
2013 vs 2012	0.624	0.521	0.747
2014 vs 2013	0.755	0.676	0.843
2015 vs 2014	0.914	0.847	0.986
2016 vs 2015	1.106	0.985	1.242
2017 vs 2016	1.339	1.110	1.614
2018 vs 2017	1.620	1.242	2.113

Wald Confidence Interval for Odds Ratios COL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	1.145	0.542	2.418
2013 vs 2012	1.047	0.624	1.756
2014 vs 2013	0.957	0.693	1.323
2015 vs 2014	0.875	0.672	1.140
2016 vs 2015	0.800	0.534	1.199
2017 vs 2016	0.731	0.393	1.362
2018 vs 2017	0.668	0.283	1.578

Wald Confidence Interval for Odds Ratios FOT			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.537	0.361	0.799
2013 vs 2012	0.703	0.529	0.935
2014 vs 2013	0.921	0.769	1.102
2015 vs 2014	1.206	1.086	1.340
2016 vs 2015	1.580	1.384	1.804
2017 vs 2016	2.070	1.649	2.600
2018 vs 2017	2.712	1.935	3.801

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.620	0.380	1.013
2013 vs 2012	0.720	0.511	1.016
2014 vs 2013	0.837	0.677	1.034
2015 vs 2014	0.972	0.943	1.120
2016 vs 2015	1.128	0.915	1.392
2017 vs 2016	1.311	0.932	1.843

Wald Confidence Interval for Odds Ratios GEN			
Label	Estimate	95% Confidence Limits	
2018 vs 2017	1.522	0.935	2.477

Wald Confidence Interval for Odds Ratios NAL			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.456	0.337	0.617
2013 vs 2012	0.554	0.450	0.682
2014 vs 2013	0.673	0.593	0.763
2015 vs 2014	0.817	0.741	0.902
2016 vs 2015	0.993	0.848	1.163
2017 vs 2016	1.206	0.942	1.545
2018 vs 2017	1.465	1.038	2.069

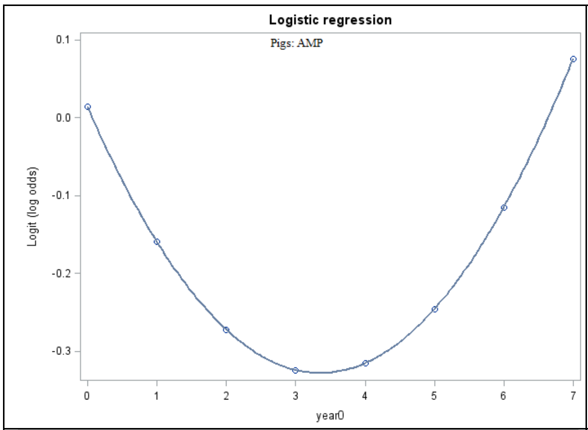
Wald Confidence Interval for Odds Ratios SMX			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.789	0.681	0.915
2013 vs 2012	0.843	0.759	0.937
2014 vs 2013	0.901	0.843	0.963
2015 vs 2014	0..963	0.920	1.007
2016 vs 2015	1.028	0.966	1.095
2017 vs 2016	1.099	0.994	1.214
2018 vs 2017	1.174	1.018	1.354

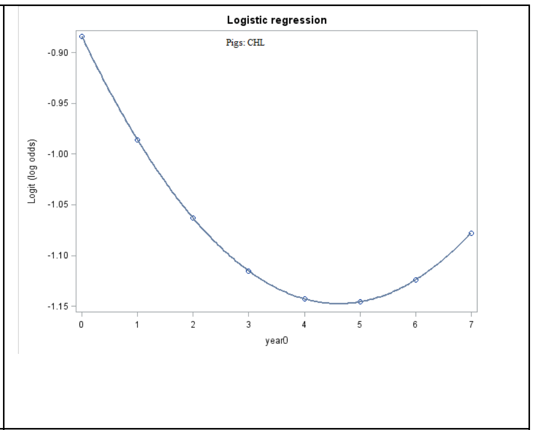
Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.540	0.368	0.792
2013 vs 2012	0.697	0.530	0.916

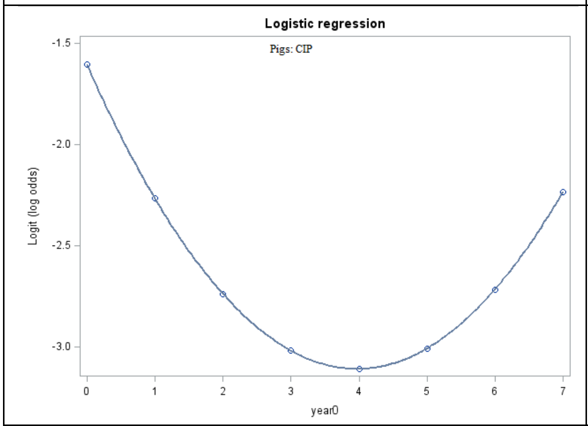
Wald Confidence Interval for Odds Ratios TAZ			
Label	Estimate	95% Confidence Limits	
2014 vs 2013	0.900	0.758	1.068
2015 vs 2014	1.162	1.050	1.286
2016 vs 2015	1.501	1.316	1.713
2017 vs 2016	1.938	1.547	2.429
2018 vs 2017	2.503	1.795	3.492

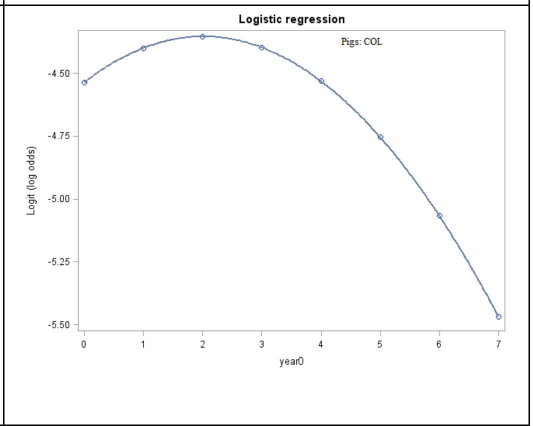
Wald Confidence Interval for Odds Ratios TET			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.768	0.644	0.915
2013 vs 2012	0.821	0.732	0.922
2014 vs 2013	0.879	0.822	0.939
2015 vs 2014	0.940	0.882	1.001
2016 vs 2015	1.005	0.901	1.122
2017 vs 2016	1.075	0.908	1.274
2018 vs 2017	1.150	0.912	1.451

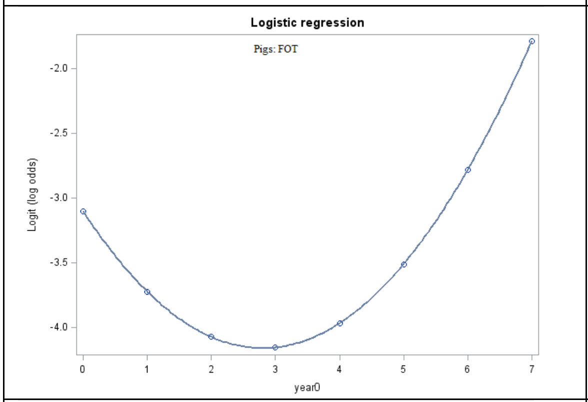
Wald Confidence Interval for Odds Ratios TMP			
Label	Estimate	95% Confidence Limits	
2012 vs 2011	0.782	0.674	0.906
2013 vs 2012	0.829	0.746	0.920
2014 vs 2013	0.879	0.822	0.939
2015 vs 2014	0.932	0.890	0.975
2016 vs 2015	0.988	0.928	1.052
2017 vs 2016	1.048	0.948	1.158
2018 vs 2017	1.111	0.964	1.281

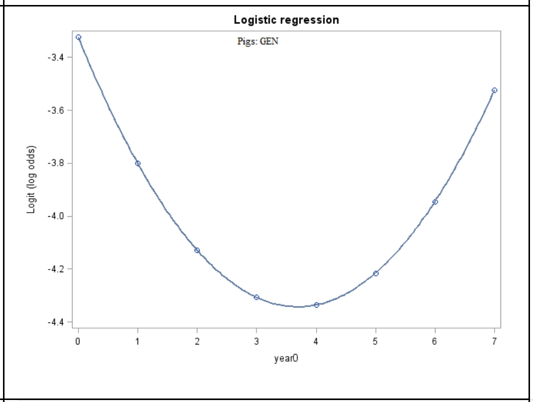

Logistic regression
Pigs: AMP
Logit (log odds)
year0

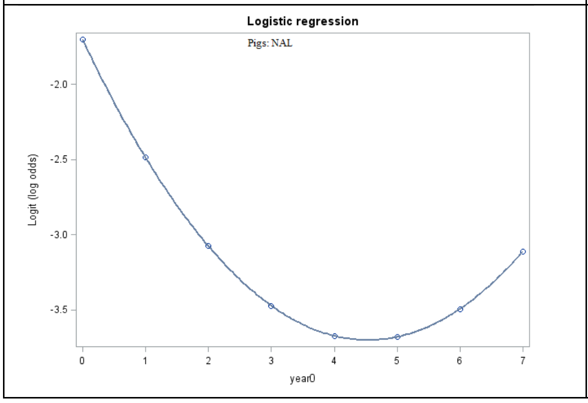

Logistic regression
Pigs: CHL
Logit (log odds)
year0

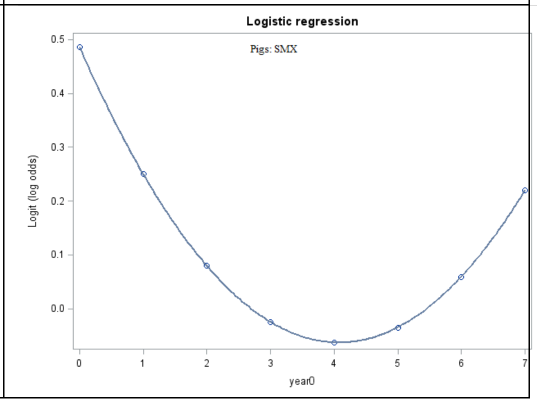

Logistic regression
Pigs: CIP
Logit (log odds)
year0

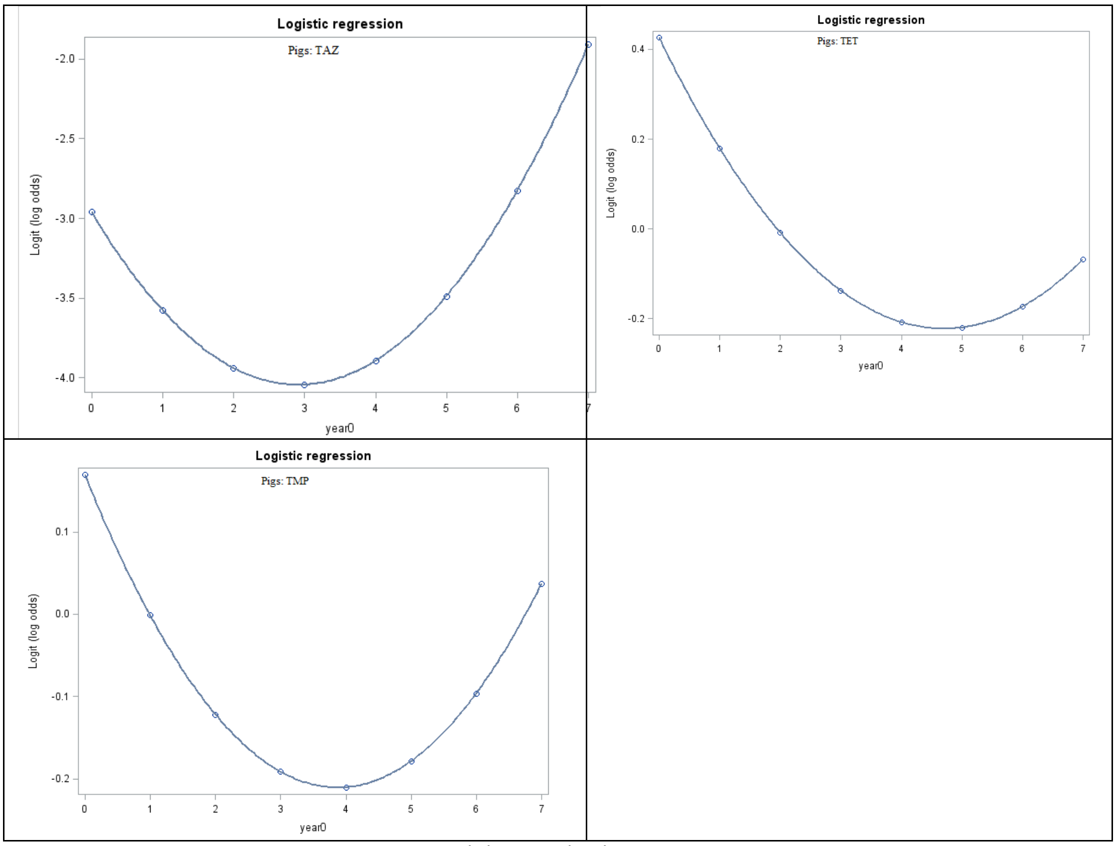

Logistic regression
Pigs: COL
Logit (log odds)
year0


Logistic regression
Pigs: FOT
Logit (log odds)
year0


Logistic regression
Pigs: GEN
Logit (log odds)
year0


Logistic regression
Pigs: NAL
Logit (log odds)
year0


Logistic regression
Pigs: SMX
Logit (log odds)
year0



Logistic regression, by years.

Legend: year0: 0=2011; 1=2012; 2=2013; 3=2014; 4=2015; 5=2016; 6=2017; 7= 2018

ANNEX 2: GEE linear model with multiple comparisons corrections (p-values)

CALVES

Test	probz	Bonferroni	Linear Stepup
AMP	0.1635	1.0000	0.1999
CHL	0.0080	0.0881	0.0220
CIP	<0.0001	0.0010	0.0005
COL	0.0008	0.0090	0.0030
FOT	0.5779	1.0000	0.6357
GEN	0.0745	0.8192	0.1170
NAL	<0.0001	<0.0001	<0.0001
SMX	0.0151	0.1666	0.0333
TAZ	0.8138	1.0000	0.8138
TET	0.1393	1.0000	0.1915

Test	probz	Bonferroni	Linear Stepup
TMP	0.0525	0.5771	0.0962

CATTLE

Test	probz	Bonferroni	Linear Stepup
AMP	0.0100	0.1095	0.0365
CHL	0.1471	1.0000	0.2023
CIP	0.0430	0.4725	0.1181
COL	0.0018	0.0193	0.0096
FOT	0.7813	1.0000	0.7873
GEN	0.0868	0.9553	0.1592
NAL	0.0004	0.0047	0.0047
SMX	0.4265	1.0000	0.5213
TAZ	0.5585	1.0000	0.6143
TET	0.0566	0.6230	0.1246
TMP	0.1468	1.0000	0.2023

CHICKEN

Test	Probz	Bonferroni	Linear Stepup
AMP	0.2518	1.0000	0.2518
CHL	0.0685	0.7531	0.0753
CIP	0.0005	0.0053	0.0018
COL	0.0011	0.0124	0.0031
FOT	0.0379	0.4174	0.0464
GEN	0.0053	0.0588	0.0098
NAL	<0.0001	<0.0001	<0.0001
SMX	0.0080	0.0880	0.0126
TAZ	0.0217	0.2386	0.0298

Test	Probz	Bonferroni	Linear Stepup
TET	<0.0001	<0.0001	<0.0001
TMP	0.0032	0.0351	0.0070

PIG

Test	probz	Bonferroni	Linear Stepup
AMP	0.6324	1.0000	0.6957
CHL	0.2568	1.0000	0.4035
CIP	0.0582	0.6404	0.1281
COL	0.3838	1.0000	0.5006
FOT	0.0003	0.0024	0.0021
GEN	0.9214	1.0000	0.9214
NAL	0.0004	0.0042	0.0021
SMX	0.1109	1.0000	0.2034
TAZ	0.0014	0.0158	0.0053
TET	0.0023	0.0255	0.0064
TMP	0.4096	1.0000	0.5006

Results of the univariate (logistic regression) and multivariate (GEE) analysis are summarized hereafter in a table using simple symbols in order to get an overall picture of the situation over the seven consecutive years and to easily make comparisons between animal categories. All indicated trends (↑, ↓) were statistically significant (p = 0.05) both in univariate (logistic regression) and multivariate (GEE) analysis, even after using correction methods for multiple testing (Bonferroni and Linear step-up method), unless otherwise mentioned.

	Veal Calves	Beef Cattle	Chickens	Pigs
AMP	++	↓1	++	
CHL	↓1		2	
CIP	↓	3	↓++	2
COL	↓	↓	↓	
FOT			↓1	↑
GEN	2		↑	
NAL	↓	↓	↓+	↓
SMX	↓1++		↓1++	+
TAZ			↓1	↑
TET	++	2	↓	↓+
TMP	+2		↓+	

++: prevalence (> 50%) for the 8 consecutive years
+: prevalence (> 40%) for the 8 consecutive years
↓ = **significant decreasing** trend of resistance
↑ = **significant increasing** trend of resistance
1=Trend not significant after p value adjustment with Bonferroni method
2= Trend significant by univariate analysis but not by multivariate analysis
3= Trend significant after p value adjustment with Linear method and with Bonferroni method

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