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Maastricht University

Faculty of Sciences
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Master of Statistics

Masterthesis

Meta-Analyse: influence of Route of antimicrobial administrationon Resistance

Cindy Lorena Baquero Portela

Thesis presented in fulfillment of the requirements for the degree of Master of Statistics, specialization Epidemiology & Public Health Methodology

SUPERVISOR :

Mevrouw Robin BRUYNDONCKX

SUPERVISOR :

Dr. Boudewijn CATRY

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Abstract

Objective: The aim of this meta-analysis was to identify the influence of route of administration on antimicrobial resistance (AR) in animals and to identify the influence of other possible factors that might be associated with it. A variety of studies were searched in English and Spanish. Studies identified as potentially relevant were assesses against eligibility criteria.

Methodology: Random-effects model was used to analyze whether the studies found a positive influence of the route of antimicrobial administration and AR. A subsequent meta-regression was conducted to assess the influence of the different factors that may be associated with AR.

Results: 1806 articles were identified, but only five were considered eligible for inclusion. The meta-analysis showed a significant pooled odds ratio of 4.57(95% confidence interval 1.43 to 14.56). From the five variables extracted from the articles, four were identified to have a positive association between the oral treatment group and AR.

Conclusions: Using a set of studies we found that oral antimicrobial administration has a higher risk of development of antibiotic resistance in comparison to the injectable administration route. A subsequent meta-regression revealed that the type of antibiotic, the animal species, the inoculated bacteria are important factors that influence the risks of having AR in the oral administration group. The limited number of studies on this topic call for more studies on the impact of oral administration on AMR development and spread in animals and humans.

Keywords: Antibiotic resistance, Antibiotic usage, meta-analysis, random-effect model.

1 Introduction

Development of antibiotics has been one of the most important medical advance of the past centuries. The introduction of antibiotics to treat infectious diseases has improved tremendously human and animal health [14]. The ability of antibiotics to treat and cure infections has led to a decrease in the fatality rate due to bacterial infections. At the same time, the overuse and misuse of antibiotics in people and animals in the past years, has produced that microorganisms such as bacteria, viruses, parasites and fungi develop genetic mutations that affect the activity of the drug [15]. The resistance of this microorganisms is a major public health problem, since this threats the capacity of the antibiotics in treating common infectious diseases, resulting in prolonged illness, disability and death [16].

Recent reports suggest that the absolute number of infections due to resistant microorganisms are increasing worldwide, with large variations between countries and bacterial species. The hope of overcoming this problem by the development of new antibiotics, is weakened by the probability that microorganisms will evolve to resistance to these new antibiotics [18].

There are different factors that can influence the antimicrobial resistance (AR). Factors related to antimicrobial usage like the amount of antibiotics used, the dosing regimen, the type of antimicrobial agent and the administration route, have been seen to influence the selection, spread and persistence of AR. The latter one, will be the main topic of this thesis but oriented in the specific case of antibiotics used in animals. Oral administration seems to have a higher selection pressure on the gastrointestinal microbiota in comparison to parenteral treatment [17].

Previous studies performed with chickens and pigs have concluded that oral administration of antimicrobial may increase the probability of AR in *E. coli* (Simoneti *et al.*2015; Vieira *et al.*, 2009). However, those studies compared treated versus untreated animals, ignoring to analyze other routes of administration. While some articles have reviewed AR specifically in oral administration, this review will not focus just on effect of oral administration of antibiotics in farm animals, but also on the effect comparison of different administration routes on resistance selection and spread.

The main goal with this thesis is to combine information of previously published data from different studies to investigate the influence of route of antimicrobial administration on resistance and also to examine which other factors can affect the resistance.

2 Methodology

2.1 Study selection and search strategy

A systematic literature search was conducted to identify randomized controlled trials that investigated the difference between injectable and oral treatment on the fecal microbiota. Sources were identified from MEDLINE, Scopus, SciELO, MEDES and PubMed databases from 2000 to 2017, only animal studies. There were no language restrictions at screening. Keywords used were "drug resistance, bacterial"[MeSH Terms] OR "drug resistance, microbial" [Mesh Terms] AND ("injections, intramuscular"[MeSH Terms] OR (injections, intravenous" [MeSH Terms] AND "administration, oral"[MeSH Terms] AND "Microbiota/drug effects*"[MeSH Terms] OR “Dose-Response Relationship, Drug” [MeSH Terms]. Sensitivity filter was used to limit studies to clinical trials. The reference lists of any studies meeting inclusion criteria were reviewed manually to identify additional relevant publications. We adhered to PRISMA (preferred reporting items for systematic reviews and meta-analyses) recommendations where possible[19].

2.2 Inclusion/Exclusion Criteria

Studies were required to meet the following criteria: (1) prospective randomized controlled design; (2) enrolled mammal individuals, irrespective of health status at the beginning of the study (3) investigation of bacteria in the digestive tract via culture, (3) at random study group that received oral and study group that received parenteral treatment with the same antimicrobial agent using the therapeutic dose regimen specified for that drug (4), negative controlled arm included adult subjects with. If studies did not provide sufficient details on microbiological counting, authors were contacted to obtain additional data.

2.3 Data abstraction and validity assessment

Data abstraction was completed by the author of this thesis, and checked for completeness and accuracy by the internal and external supervisors. Where data were not reported, authors were contacted to provide additional data. If we received no response, graphs were used to estimate data using WebPlotDigitizer 4.1 .

2.4 Study outcome measures

The primary outcome was the difference in occurrence of antimicrobial resistance organisms following either injectable or oral exposure of antimicrobial agents. The highest difference within each study was assessed in order to model the worst case scenario. Secondary outcomes were the time at what the difference occurred.

Since antibiotic resistance is the result of a complex interaction of many factors, like antimicrobial drugs, bacterias, host and environment [21], explanatory variables were extracted from the studies to investigate the influence of these variables on the effect estimate. These variables are listed on table 1.

Table 1: Explanatory variables for meta-analysis to study the effect of route of administration on antimicrobial resistance

Variable	**Categories**
Animal species	Calves Pigs Mice Chickens
Isolated bacteria	*E. coli* *Enterococcus* *Salmonella*
Antibiotics used	Tetracycline Enrofloxacin Ampicillin
Year of publication	
Period of antibiotic administration	1st period 2nd period 3rd period

The duration of antibiotic administration has been split for this study into 3 different time intervals in the variable *Period of antibiotic administration*, where period 1 corresponds to the first third of the time of treatment, period 2 to the subsequent third, and period 3 to the last one. The effect size used for this meta-analysis was the odds-ratio, which was calculated for all the studies with the extracted information. Two data set (implemented in Microsoft excel; 2016) were created from these data. The first data set (Table

4) contains information regarding the difference in occurrence of antimicrobial resistance organisms where the highest difference within each study was assessed. The second data set (Table 5) contains information regarding the study periods and other parameters used in the in further analysis as the meta-regression.

Some studies have reported information on AR from more than one antibiotic and one bacterium. On these cases, the extra information has been considered on the meta-analysis as an independent study.

2.5 Statistical analysis

2.5.1 Meta-analysis

Meta-analysis is a statistical method that combines information from several independent research studies with the aim to estimate a single and more precise estimate of interest. The purpose of meta-analyses are to increase statistical power; to summarize the findings when several individual studies disagree; and to improve the estimates of an effect size to allow more solid conclusions. Meta-analysis plays an essential role in evidence-based medicine, where, according to the hierarchy of evidence, meta-analysis (specially those using randomized controlled trials (RCTS)) are considered to be superior [2].

In the meta-analysis the important outcomes (known as effect size) are computed for each study to later to be able to aggregate them, to asses the consistency of the effects across the study and compute a common effect size[3]. Depending on the type of study and the information provided in each of them, different outcomes can be used for a meta-analysis, including risk ratios, risk differences, odds ratios, (standardized) mean difference and correlations.

There are specific statistical methods that are used in meta-analysis to combine the information. The data from individual studies can be analyzed using either of two models: the *fixed-effects model* or the *random-effects model*. Under the fixed-effects model we assume that there is no heterogeneity between the effect sizes; the studies are assumed all to be estimating a single true effect size[4] and that the differences in the observed effects are due to sampling errors[5]. On the other hand, the random-effects model is more conservative, this model allows that the true effect can vary between studies, and takes into account the extra variation implied in making this assumption. Due to expected clinical heterogeneity between the studies, the random-effects model was used because (as discussed above) it allows to incorporate into the model the heterogeneity among the true effects.

2.5.2 Random-effects model

When meta-analyzing effect sizes from different studies, we expect them to have enough characteristics in common to be able to combine their information for statistical inference[7], however, integrating findings from different studies is one of the major difficulties, due to the diverse nature of the studies. For a meta-analysis the studies can differ, in terms of patient characteristics or methods used[6], however, choosing the right model that captures this variability across the studies is important to avoid misleading inferences about treatment effects.

The random-effects model assumes that there is no single true population or effect across the studies. Rather assumes that there is a distribution of the effects with a fixed mean and variance. Therefore, the observed variability in sample estimates of effect size is partially due to the variability in the underlying population parameters and partially due to the sampling error of the estimator about the parameter value[7]. The random effects-model can be written as:

$$\theta_i = \mu + u_i + \epsilon_i$$

where $u_i \sim N(0, \tau^2)$ describes the between-study variation and $\epsilon_i \sim N(0, v_i)$ describes the within-study variation. v_i and ϵ_i are assumed to be independent and then, the random-effects model can be re-written as:

$$\theta_i \sim N(\mu, \sigma^2 + \tau^2)$$

where τ^2 is the inter-study variance and represents both the degree to which true treatments effects vary across studies as well as the degree to which individual studies give biased assessments of treatment effects[6]. σ^2 is the intra study variance which reflects within-study sampling variance.

Estimation of the overall mean effect size
The first step in a random-effects meta-analysis is to calculate an estimate for the inter-study variance τ^2 to be able to calculate the overall effect size and its standard error[6]. Suppose $s_1^2, ...s_n^2$ and t^2 are the estimates for σ^2 and τ^2 respectively. We get the following estimate for μ

$$m_w = \frac{\sum_{i=1}^{k} w_i y_i}{\sum_{i=1}^{k} w_i}$$

where

$$w_i = \frac{1}{(t^2 + s^2)}$$

and the estimated standard error of the summary effect would be

$$s.e.(m_w) = \frac{1}{(\sum_i w_i)^{1/2}}$$

The last expression for $s.e.(m_w)$ is a conditional standard error under the assumption that the estimates $s_1^2, ...s_1^2$ and t^2 are equal to the true variances $\sigma_1^2, ...\sigma_1^2$ and τ^2 respectively. The expression for the standard error is an underestimate of the true standard error of m_w[6].

Estimation of the inter-study variance τ^2
There are several methods to estimate τ^2. Each of them have different properties in terms of bias, mean squared error (MSE) and efficiency. For our case the Paule and Mandel (PM) method for estimate τ^2 was implemented, since this approach is more robust when normality assumption does not hold[6]. The Paule and Mandel estimating equation can be defined as:

$$Q_{gen} = \sum_i w_i \{y_i - m_w\} \sim X_{k-1}^2$$

The solution $t_{opt}^2$is obtained by a numerical iteration starting with $t^2 = 0$. Then, t_{PM}^2 is defined as t_{PM}^2= max (0,t_{opt}^2)[8]. PM is an iterative estimator that belongs to the family of the Generalized method of moments (GMM) estimators, where the random-effects model weights and overall effects are simultaneously calculated using the true value of t^2 that is part of the important quantity[9].

Correlated random time effects model
This model proposed by Musekiwa *et. al.*, is an extension of the random-effects model. In this model we assume a heteroscedastic AR(1) covariance structure for the random

period effect, where the within-study serial correlations between longitudinal effect sizes are assumed to be zero. So then, the variance-covariance matrix is given by

$$\begin{bmatrix} \tau_1^2 & \rho\tau_1\tau_2 & \rho^2\tau_1\tau_3 \\ & \tau_2^2 & \rho\tau_2\tau_3 \\ & & \tau_3^2 \end{bmatrix}$$

As a result, the dependence between effect sizes become stronger for time points that are closer to one another.

The random time effects model can also include one or more predictors (also called moderators), that can capture a part (or fully) the heterogeneity in the true effects that has not been captured in a model without them. A model with predictors then is given by

$$\theta_i = \beta_0 + \beta_1 X_{i1} + ... + \beta_{p'} X_{ip'} + u_i + \epsilon_i$$

where x_{ij} denoted the value of the jth moderator variable for the ith study with associated regression parameter β_j where β_0 is the global effect size. We assume that u_i is again $u_i \sim N(0, \tau^2)$, where τ^2 denotes the amount of residual heterogeneity that is not accounted by the study variables in the model[10].

2.5.3 Publication bias

Publication bias is one of the main concerns in a meta-analysis, and could arise when only studies with positive statistical significance tend to be published. Therefore all published studies may be selected for a meta-analysis and the resulting inferential results may be biased [10]. Publication bias has traditionally been assessed using funnel plots whereby the estimates from all studies are plotted against their standard error. A symmetrical funnel plot suggests no publication bias, however a skewed funnel plot is an indicator of potential publication bias.

Funnel plots are a useful graphical tool for diagnosing certain forms of publication bias, yet, it is important to be able to test this asymmetry mathematically. Various tests have been suggested in the literature. For this meta-analysis, the Egger's test was used. This approach works by fitting the points by linear regression. In the case of a symmetrical funnel plot, the intercept on the X-axis should be close to zero, whereas with asymmetry it may deviate considerably from zero[20].

2.6 Software

2.6.1 The R Package: metafor

The `metafor` package is a special `R` package for conducting meta-analysis. This package contains different functions for fitting meta-analytic fixed and random-effects models and allows the inclusion of study variables[13]. This package also allows various to plot functions for assessing the model fit, diagnoses and tests for publication bias.

To calculate the effect size estimates and their corresponding sampling variance, `metafor` package has the `escalc()` function. This function allows to calculate the most commonly used effect sizes in meta-analysis (Odds ratios, Relative Risks, etc.).

To perform the analysis of the primary and the secondary outcome, the `rma()` function was used. In the case for the correlated random time effects model the function `rma.mv()` was utilized and the random effects structure was specified using the random argument through the formula `rma()` =~factor(period) | factor(study ID). So, the effects with different study ID are assumed to be independent, while effects with the same the study ID share correlated random effects corresponding to the levels of the period variable.

3 Results

3.1 Search results

The literature search identified 1806 citations. After exclusion of articles based upon review of the title and abstracts further exclusion was done after careful review of the full text. The studies that were eligible for meta-analysis are presented in Table 4.

3.2 Meta-analysis

Figure 1 shows the forest plot for the meta-analysis in which the antimicrobial resistance in oral and injectable administration routes was assessed in the worst case scenario. The meta-analysis revealed that the oral route has a significant positive relationship in antibiotic resistance, with a pooled effect size (Odds Ratio) of 4.57 (z=2.56, *p-value*=0.01, 95% CI= 1.43-14.56).

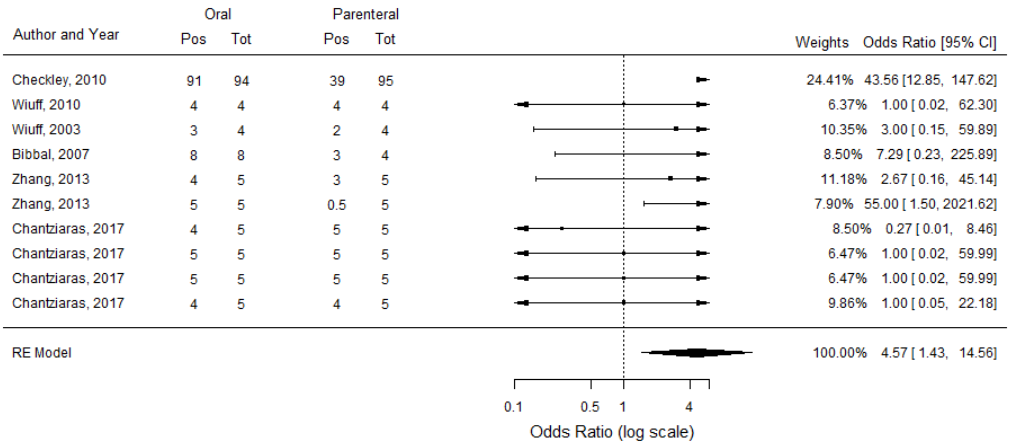

Figure 1: Forest plot (random-effects meta-analysis) showing odds ratio and 95% confidence interval for each study along with study weight.

This figure also displays the weight and the odds ratio for each study. We can note that one of the studies (Checkley et. al 2010) has a higher weight in comparison with the other studies. To ensure that the sample size of this study does not influentiate in our conclusions, we performed again the analysis by reducing its sample size to 10 (to make it comparable in size with the rest of the studies) but keeping the proportion of the resistant in the oral and injectable group. The results obtained showed that the overall effect estimate becomes smaller, and is now on the borderline of statistical significance. However, it is important to notice that the estimate did not change its direction, and we still can conclude that the oral route has a positive relationship in antibiotic resistance. The forest plot for this analysis with the reduced sample size is shown in figure 7 in the appendix section.

3.2.1 Influential case diagnosis

An influential case diagnosis was performed to check the presence of influential studies on the results of the meta-analysis. Figure 2 shows the plots of some diagnostic measures, which suggests that studies 1 (Checkley 2010) and 7 (Chantziaras 2017) introduce some

additional residual heterogeneity into the meta-analysis. However, study 1 (Checkley 2010) seems to be the most influential study.

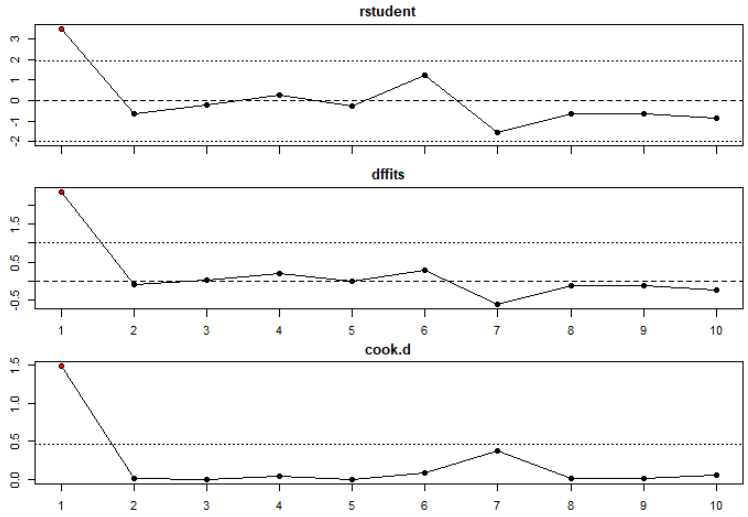

Figure 2: Plot of standardized residuals, DFFITS values and Cook's distance as assessed during a meta-analysis on the effect of route of administration on antimicrobial resistance.

3.2.2 Heterogeneity analysis

Significant heterogeneity was observed among the true effects, since the estimated total amount of heterogeneity τ^2 was 1.0450 and the percentage of total variability due to heterogeneity was 32.39%. Furthermore the Test for Heterogeneity was statistically significant with Q=17.83,*p-value*=0.037, indicating that there is more variability in the effect sizes than may be expected.

From the funnel plot in figure 3, we can note that the study of Checkley *et al.* is one of the studies with the largest odds ratio and the largest sample size. Testing for asymmetry the Egger's test suggest that there is a large degree of asymmetry (Egger's test *P-value*=0.0003), however, this is possibly due to the influence of the Checkley *et al.* study. To confirm this, we have performed the analysis once again with the scaled study. The results obtained showed that there is longer no asymmetry in the funnel plot (Egger's test *p-value*=0.74).

3.3 Univariate random-effects meta-analysis for period 1, 2 and 3

An univariate random-effects meta-analysis for period 1, 2 and 3 was performed. The results obtained are summarized in table 2 and the forest plots in figures 4, 5 and 6.

Table 2: Estimates univariate meta-analysis for period 1, 2 and 3

	Odds ratio (CI)	*p-value*	Q-test(*p-value*)
Period 1	4.22 (1.22-14.7)	0.023	5.41(0.79)
Period 2	2.97 (1.72-5.13)	<0.0001	6.60(0.67)
Period 3	2.8 (1.59-5.13)	0.0004	20.07(0.01)

The results in table 2 show that the odds of antimicrobial resistance in the oral administration group were significantly higher than in the parental group. This result was

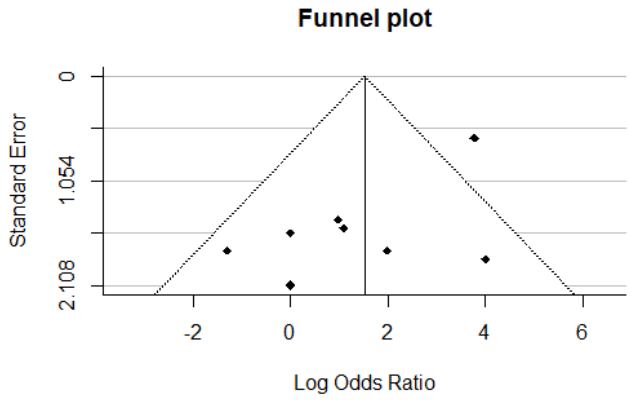

Figure 3: Funnel plot

consistent for all three periods. The highest odds ratio was observed in period 1 and decreases in the subsequent periods. All the three odds ratios for period 1, 2 and 3 were statistically significant. Significant heterogeneity was observed among the true effects.The heterogeneity test was only significant for period 1 (Q = 20.0746, p-val = 0.0175).

Figure 8 shows the residuals for all the periods of antibiotic administration. We can note that the study of Checkley *et al.* seems to be influential for period 1 and 3. On the other hand, the study of Zhang *et al.* seems to have this effect on period 2.

The funnel plots for all the three periods are shown in figure 9. These plots together with the test of asymmetry suggest that period 1 has some level of asymmetry (Egger's test=-2.27, *P-value*=0.02). However, any of the tests suggest asymmetry in the respective funnel plots for period 2 and 3 of antibiotic administration.

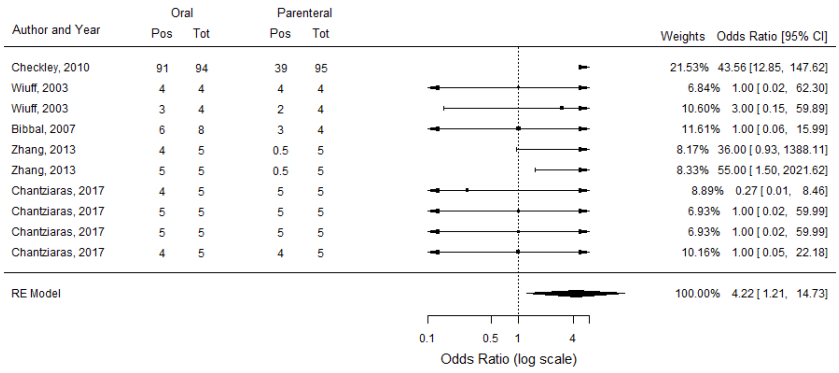

Figure 4: Forest plot period 1

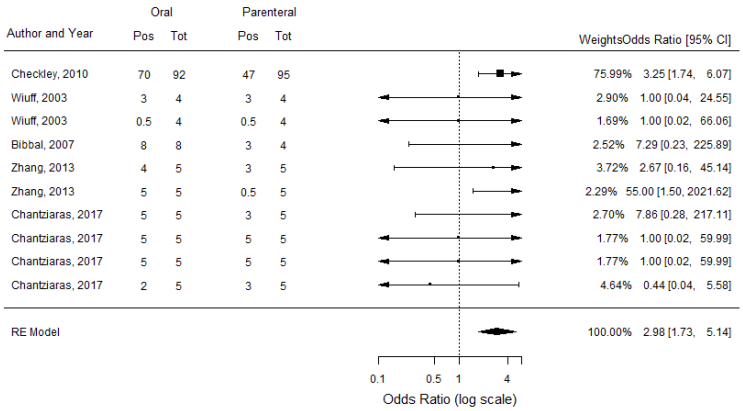

Figure 5: Forest plot period 2

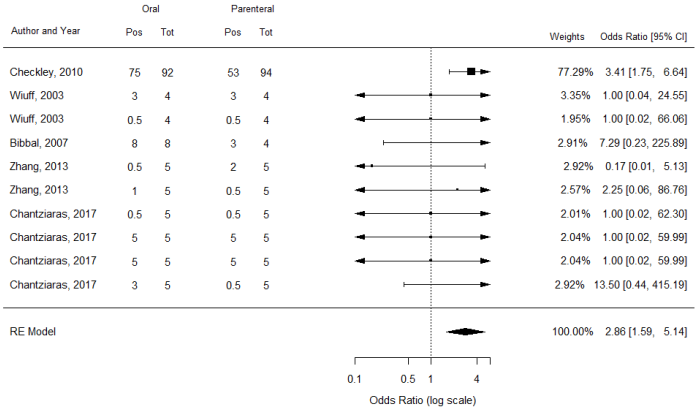

Figure 6: Forest plot period 3

3.4 Random time-effect model

Due to the high correlations between the dependent variables (Table 6), univariate models were fitted. Furthermore, to ensure that the heterogeneity caused by the study of Checkley *et al.* does not affect our conclusions, we have performed an additional analysis with the scaled study. On this extra analysis, the sample size of the mentioned study was scaled up to a size comparable with the size of the rest of the studies, keeping the proportions of the study with the original sample size. The results obtained for the meta-regression in the scaled analysis are shown in table 7 in the appendix section. The meta-regression for the variable *period* in the full size analysis, showed that in the second and third period of antimicrobial administration is more likely to develop AR in the oral group than in the injectable one. When we look at the results for the scaled analysis, we observe that the effect estimates become smaller, and is no longer statistically significant. Regarding the test for residual heterogeneity is not significant in both cases, possibly indicating that it is not necessary to include more moderators in the model.

The results obtained for the meta-regression of the variable *antibiotics* in the full size analysis suggest that tetracycline and ampicillin have a positive effect on antimicrobial resistance in the oral group. For the scaled analysis, the ampicillin remained significant, on the contrary the tetracycline is now on the borderline of statistical significance. The test for residual heterogeneity in both analysis was once again not significant.

Results obtained for the variable *bacteria* showed that *E. coli* might have a positive association with antimicrobial resistance in the oral group than in the injectable group, nevertheless, comparing the results obtained in the scaled analysis, this bacterium is no longer significant, possibly indicating that the sample size of the Checkley *et al.* study influenced on the result.

The meta-regression for the variable *animal* in the in the full size analysis, calves suggested that calves are more likely to have antimicrobial resistance in the oral group than in the parenteral group. In the scaled analysis, however, the mice were also statistically significant. The heterogeneity test in both analysis, was not significant, possibly indicating that it is not necessary to include more moderators in the model. The variable *year of publication* did not have any influence on antimicrobial resistance in the oral and injectable group.

Table 3: Estimates of the univariate random time-effect model

Categorical Variable	**Odds Ratio (CI)**	**P-value**	**QE-Test***	**QM-Test****
Animal			30.17 (0.26)	31.75 (< .0001)
Pigs	1.89 (0.52-6.90)	0.33		
Mice	3.81 (0.81-17.86)	0.08		
Chickens	1.44 (0.44-4.78)	0.54		
Calves	3.40 (2.15-5.36)	<0.0001*		
Bacteria			35.15 (0.13)	14.42 (0.002)
Salmonella	1.07 (0.17-6.55)	0.94		
Enterococcus	3.01 (0.55-16.57)	0.20		
E.coli	2.26 (1.49-3.97)	0.0003*		
Antibiotics			29.61 (0.33)	32.39 (< .0001)
Tetracycline	3.20 (2.05-5.01)	<.0001*		
Enrofloxacin	1.33 (0.50-3.54)	0.56		
Ampicillin	7.40 (1.60-34.31)	0.01*		
Year of publication	0.99 (0.84-1.70)	0.96	37.25 (0.11)	0.0005 (0.98)
Period of administration			32.09 (0.22)	11.68 (0.008)
1st period	5.37 (0.83-34.72)	0.07		
2nd period	2.43 (1.12-4.42)	0.021*		
3rd period	2.49 (1.34-4.61)	0.003*		

*Test for Residual Heterogeneity
**Test of Moderators

Due to the variability and the high correlation between the variables, they could not be included into a single model. Moreover, the moderator analysis has found that all the variables, except for *year of publication*, have an influence on antimicrobial resistance in the oral administration group.

4 Discussion

Antimicrobials play an important role in animal and human health. In the past years access to antibiotics has increased and its use has been widespread. Unfortunately, the antimicrobials misuse has lead to a significant increase of number of bacterias resistant to the different antibiotics available in the market.

One of the most debated use of these antibiotics is in the food animal production. The majority of antimicrobials agents are given to animals for prophylaxis and metaphylaxis by oral administration [22]. This practice has (beside other risk factors) an increased risk of bacterial resistance.

In the present thesis, despite the great variability and the small number of animals studied in each treatment group, the results from this meta-analysis revealed that subjects who received oral antimicrobials were more likely to have AR in comparison with the animals that received antimicrobials in the injectable route. Most of the studies used for this meta-analysis agree that the use of feed or water antimicrobials is associated with a high risk of development of AR regardless of the species. This might be associated with a disturbance in the gut microbiota occasioned by the administration of oral antibiotics, leading to a high excretion of resistance strains in feces.

It feels needed to remark that, even though the study of Chantziaras have found significant effect on the administration route, we did not find such a difference between administration routes when analyzing this article data. The reason behind this mismatch is the fraction of his data that has been extracted for the meta-analysis. While the scope of the Chantziaras article includes different treatment doses of antimicrobial given to the animals (proper, double and half doses), this meta-analysis just considered the information related to the animals treated with proper doses. In this way, it was possible to compare the overall data that comes from similar doses of antibiotics given to the animals. This leads to irrelevant analysis of the extracted data when is analyzed just by its own, but when merged with the rest of the data in the meta-analysis helps to define the right conclusions.

The resistance percentage and resistance levels of bacteria in the two administration routes peaked 1 day after drug administration. The differences in the resistance levels between oral and injectable route were seen immediately, being the oral route the one with the highest percentage of resistance in most of the studies. This difference remained even though in the two subsequent periods the levels of resistance decreased gradually. Recent evidence has demonstrated that antibiotic-resistant strains can persist in gut in the absence of selective pressure [23]. In the study of Checkely *et al.* where fecal samples were taken until day 210 after the antimicrobial administration; and in that period resistant strains were still identified in the fecal samples.

The results in our meta-regression found that tetracycline and ampicillin were more likely to develop AR in the oral group. However, it is important to notice that the antimicrobial influence over AR, may be (among other reasons that we may ignore) due to the excretion routes. For example, the quinolones (which this case were not found to have any effect on antimicrobial resistance) are eliminated by renal and nonrenal routes [26], which made it difficult to differentiate the impact of the administration route in this meta-analysis.

Potential limitations should also be noted for this meta-analysis. The number of articles available were limited, and the sample size for most of them was small. Therefore, the analysis showed some variability, and with some analysis we faced lack of statistical power. Additionally, the difficulty to extract the information from some articles, where the information was not clear or was not contented in the narrative, it was necessary to extract it from the graphs, even though we use an online tool to avoid interpretation bias, some misinterpretation could have occurred.

All the authors were contacted in order to get the original raw data; we did not receive

sufficient data in due time for this meta-analysis.

Further studies are needed to assess the impact of more antibiotics and more bacterias. Additionally, studies done in humans are needed as well to assess the impact of antimicrobial administration route and AR.

5 Appendix

5.1 Studies included in the meta-analysis

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5.2 Tables and graphs

Table 4: Study details. Parameters, derived from the extracted information to perform the meta-analysis

ID	Study	Antibiotic	Bacteria	oralpos	oraltot	parentpos	parenttot	Animals	Year of publication
1	Checkley	Tetracycline	E coli	91	94	39	95	Calves	2010
2	Wiuff	Enrofloxacin	Salmonella	4	4	4	4	Pigs	2010
2	Wiuff	Quinolone	Salmonella	3	4	2	4	Pigs	2003
3	Bibbal	Ampicillin	E coli	8	8	3	4	Pigs	2007
4	Zhang	Tetracycline	Enterococcus	4	5	3	5	Mice	2013
4	Zhang	Ampicillin	Enterococcus	5	5	0,5	5	Mice	2013
5	Chantziaras	Enrofloxacin	E coli	4	5	5	5	Chickens	2017
5	Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	2017
5	Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	2017
5	Chantziaras	Enrofloxacin	E coli	4	5	4	5	Chickens	2017

Table 5: Study characteristic variables used in the meta-regression of the influence of antibiotic route administration in antibiotic resistance.

Study	Antibiotic	Bacteria	êxtbforalpos	oraltot	parentpos	parenttot	Animals	period	Year
Checkley	TCY (tetracycline)	E coli	91	94	39	95	Calves	1	2010
Checkley	TCY (tetracycline)	E coli	70	92	47	95	Calves	2	2010
Checkley	TCY (tetracycline)	E coli	75	92	53	94	Calves	3	2010
Wiuff	Enrofloxacin	Salmonella	4	4	4	4	Pigs	1	2003
Wiuff	Enrofloxacin	Salmonella	3	4	3	4	Pigs	2	2003
Wiuff	Enrofloxacin	Salmonella	3	4	3	4	Pigs	3	2003
Wiuff	Quinolone	Salmonella	3	4	2	4	Pigs	1	2003
Wiuff	Quinolone	Salmonella	0,5	4	0,5	4	Pigs	2	2003
Wiuff	Quinolone	Salmonella	0,5	4	0,5	4	Pigs	3	2003
Bibbal	AMP(ampicillin)	E coli	6	8	3	4	Pigs	1	2007
Bibbal	AMP(ampicillin)	E coli	8	8	3	4	Pigs	2	2007
Bibbal	AMP(ampicillin)	E coli	8	8	3	4	Pigs	3	2007
Zhang	TCY (tetracycline)	Enterococcus	4	5	0,5	5	Mice	1	2013
Zhang	TCY (tetracycline)	Enterococcus	4	5	3	5	Mice	2	2013
Zhang	TCY (tetracycline)	Enterococcus	0,5	5	2	5	Mice	3	2013
Zhang	AMP(ampicillin)	Enterococcus	5	5	0,5	5	Mice	1	2013
Zhang	AMP(ampicillin)	Enterococcus	5	5	0,5	5	Mice	2	2013
Zhang	AMP(ampicillin)	Enterococcus	1	5	0,5	5	Mice	3	2013
Chantziaras	Enrofloxacin	E coli	4	5	5	5	Chickens	1	2017
Chantziaras	Enrofloxacin	E coli	5	5	3	5	Chickens	2	2017
Chantziaras	Enrofloxacin	E coli	0,5	5	0,5	5	Chickens	3	2017
Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	1	2017
Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	2	2017
Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	3	2017
Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	1	2017
Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	2	2017
Chantziaras	Enrofloxacin	E coli	5	5	5	5	Chickens	3	2017
Chantziaras	Enrofloxacin	E coli	4	5	4	5	Chickens	1	2017
Chantziaras	Enrofloxacin	E coli	2	5	3	5	Chickens	2	2017
Chantziaras	Enrofloxacin	E coli	3	5	0,5	5	Chickens	3	2017

Figure 7: Forest plot (random-effects meta-analysis) for the reduce sample size study 1 (Cherckley 2009)

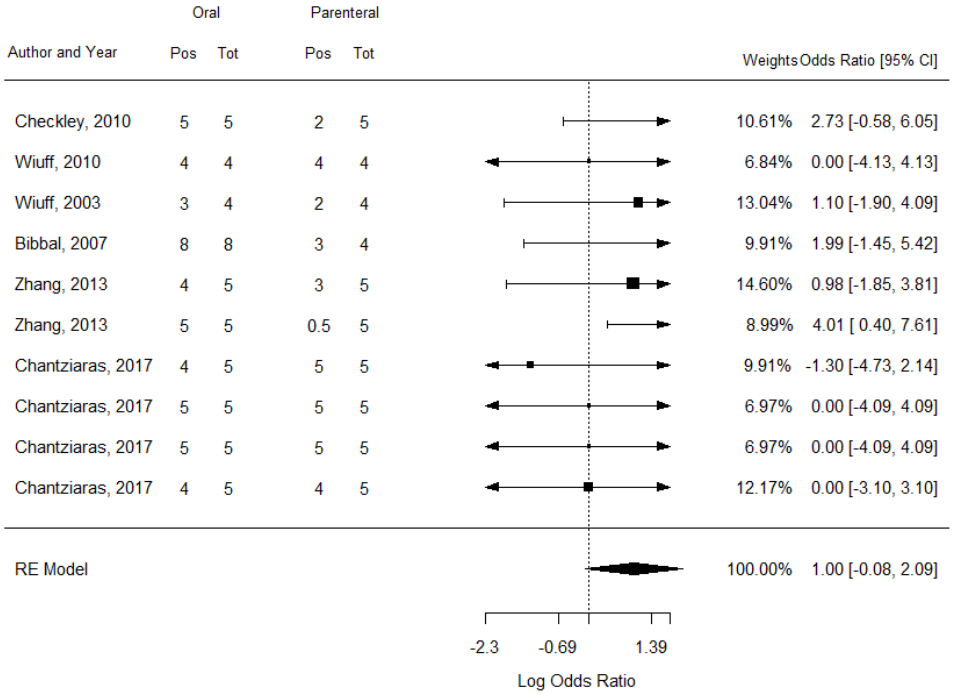
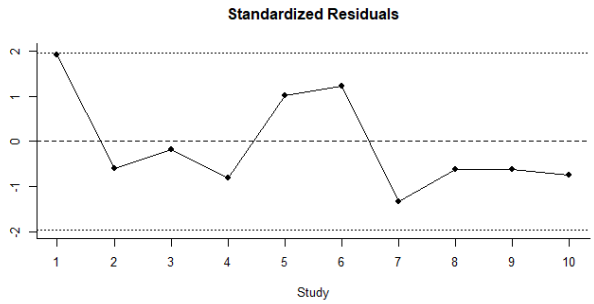
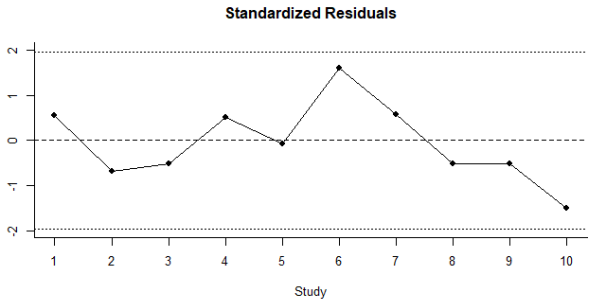


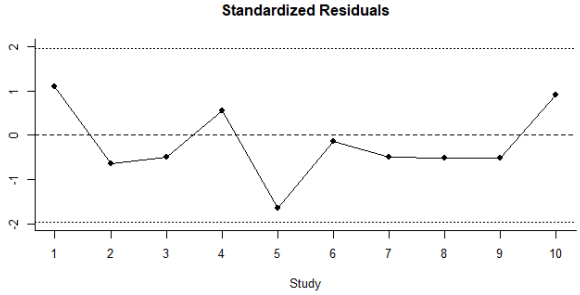
Figure 8: Standardized residuals for periods of antibiotic treatment 1, 2 and 3



(a) Standardized residuals Period 1

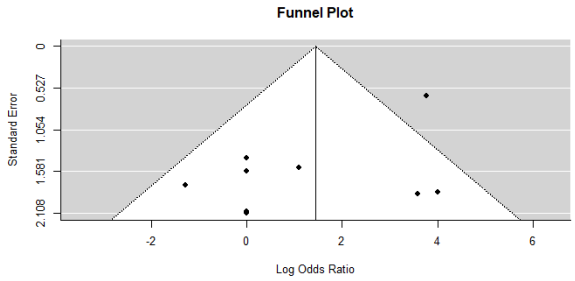


(b) Standardized residuals Period 2

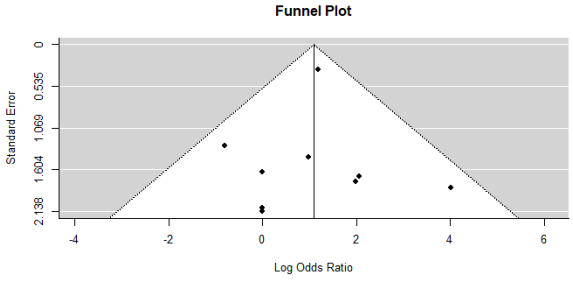


(c) Standardized residuals Period 3

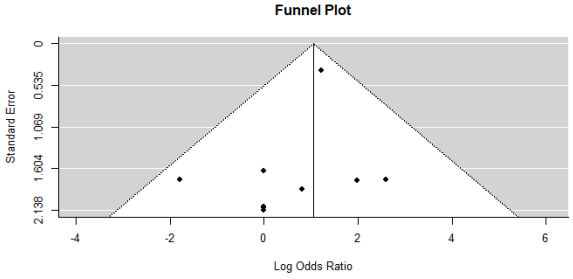
Figure 9: Funnel plots for periods of antibiotic treatment 1, 2 and 3



(a) Funnel plot Period 1



(b) Funnel plot Period 2



(c) Funnel plot Period 3

Table 6: Correlation matrix of the explanatory variables. The strength of association was calculated using Cramer's V. All the associations were highly significant.

	Antibiotic	Bacteria	Animal	Year	Period
Antibiotic	1.00				
Bacteria	0.47	1.00			
Animal	0.72	0.88	1.00		
Year	0.86	1	1	1.00	
Period	0	0	0	0	1.00

Table 7: Estimates of the univariate random time-effect model

Categorical Variable	**Odds Ratio (CI)**	**P-value**	**QE-Test***	**QM-Test****
Animal			15.81 (0.94)	12.45 (0.01)
Pigs	1.82 (0.55-5.96)	0.32		
Mice	7.89 (1.80-34.51)	0.006*		
Chickens	1.09 (0.36-3.29)	0.88		
Calves	6.28 (1.02-38.44)	0.04*		
Bacteria			17.87 (0.90)	6.79 (0.07)
E.coli	2.26 (0.82-6.18)	0.11		
Enterococcus	1.78 (0.07-3.48)	0.04*		
Salmonella	1.29 (0.23-7.38)	0.77		
Antibiotics			15.26 (0.96)	10.85 (0.01)
Tetracycline	3.46 (0.95-12.52)	0.05		
Enrofloxacin	1.27 (0.52-3.10)	0.59		
Ampicillin	6.97 (1.68-28.93)	0.007*		
Year of publication	1.00 (0.96-1)	0.46	20.38 (0.88)	0.005(0.94)
Period of administration			19.98 (0.83)	4.45 (0.21)
1st period	3.53 (0.66-18.66)	0.13		
2nd period	2.85 (0.83-9.73)	0.09		
3rd period	1.70 (0.55-5.27)	0.35		

*Test for Residual Heterogeneity
**Test of Moderators

5.3 Codes

```
setwd("C:/Users/ekaib/Desktop/Lorena/Thesis")
library(readxl)
library(metafor)
library(vcd)

###adding the data set
Analisys1 <- read_excel("C:/Users/ekaib/Desktop/Lorena/Thesis/Analisys12.xlsx")
View(Analisys1)
attach(Analisys1)

#calculating the effect sizes OR
ex2 <-escalc(ai=oralpos, ci=parentpos, n1i=oraltot, n2i=parenttot, add=1/2, to="only0",
measure= "OR", data=Analisys1, append=TRUE)
View(ex2)
```

```
###################
###Meta-analysis###
###################
FEM2<-rma(yi, vi, data=ex2, method="PM")
summary(FEM2)

##Forest plot
forest.rma(FEM2, showweights=TRUE, slab = paste(ex2$Study, ex2$Year, sep = ", "),
xlim = c(-13,8), at=log(c(0.10,0.5,1,4,6)),
ilab = cbind(ex2$oralpos,ex2$oraltot,ex2$parentpos,ex2$parenttot),
           ilab.xpos = c(-9,-8,-6,-5),cex = 0.95)
op<-par(cex=0.8,font=1.5)
text(c(-9,-8,-6,-5),11.7,c("Pos","Tot","Pos","Tot"))
text(c(-8.50, -5.40), 12.7, c("Oral", "Parenteral"))
text(-11.7,11.3,"Author and Year",pos = 3)
text(4.9,11.5,"Weights", pos=2)
text(8,11.5,"Odds Ratio [95% CI]", pos=2)
par(op)

##Analysis of the influential study##
leave1out(FEM2)
plot(inf, plotdfbs = T)

##funel plot
funnel<-funnel(FEM2, main= "Funnel plot", back="white", refline=0,
shade="white",pch=18, hlines="gray")

#to test funnel plot
regtest(FEM2, model="rma")

############################
###meta-analysis period1###
############################
library(readxl)
dat <- read_excel("Per1.xlsx")
attach(dat)
View(dat)

##creating dummy variables
dat$bac <- 0
dat$bac[dat$Bacteria =="E coli"] = 0
dat$bac[dat$Bacteria =="Salmonella"] = 1
dat$bac[dat$Bacteria == "Enterococcus"] = 2

dat$ant <- 0
dat$ant[dat$Antibiotic =="TCY (tetracycline)"] = 0
dat$ant[dat$Antibiotic =="Enrofloxacin"] = 1
dat$ant[dat$Antibiotic == "AMP(ampicillin)"] = 2

dat$anim <- 0
dat$anim[dat$Animals =="Pigs"] = 0
```

```
dat$anim[dat$Animals =="Mice"] = 1
dat$anim[dat$Animals == "Chickens"] = 2
dat$anim[dat$Animals == "Calves"] = 3

##getting the effect size and variance
ex3 <-escalc(ai=oralpos, ci=parentpos, n1i=oraltot, n2i=parenttot,
measure= "OR", data=dat, append=TRUE)
View(ex3)

# random/mixed-effects.
res <- rma(yi, vi, data = ex3, method = "PM")

##funel plot
funnel<-funnel(res, main= "Funnel plot", back="white", refline=0,
shade="white",pch=18, hlines="gray")

#to test funnel plot
regtest(res, model="rma")

##Analysis of the influential study##
leave1out(res)
plot(res, plotdfbs = T)

############################
###meta-analysis period2###
############################
##Importing the complete data set
per2 <- read_excel("Per2.xlsx")

##getting the effect size and variance
ex3 <-escalc(ai=oralpos, ci=parentpos, n1i=oraltot, n2i=parenttot,
measure= "OR", data =per2, append=TRUE)
View(ex3)

# random/mixed-effects.
res <- rma(yi, vi, data = ex3, method = "PM")

##funel plot
funnel<-funnel(res, main= "Funnel plot", back="white", refline=0,
shade="white",pch=18, hlines="gray")

#to test funnel plot
regtest(res, model="rma")

##Analysis of the influential study##
leave1out(res)
plot(res, plotdfbs = T)

############################
###meta-analysis period3###
############################
```

```
##Importing the complete data set
per3 <- read_excel("per3.xlsx")

##getting the effect size and variance
ex3 <-escalc(ai=oralpos, ci=parentpos, n1i=oraltot, n2i=parenttot,
measure= "OR", data =per3, append=TRUE)
View(ex3)

# random/mixed-effects.
res <- rma(yi, vi, data = ex3, method = "PM")

##funel plot
funnel<-funnel(res, main= "Funnel plot", back="white", refline=0,
shade="white",pch=18, hlines="gray")

#to test funnel plot
regtest(res, model="rma")

##Analysis of the influential study##
leave1out(res)
plot(res, plotdfbs = T)

######################
##metaregression#####
######################
##Importing the complete data set
dat <- read_excel("C:/Users/ekaib/Desktop/Lorena/Thesis/Data analysis2.xlsx")
attach(dat)

##getting the effect size and variance
ex3 <-escalc(ai=oralpos, ci=parentpos, n1i=oraltot, n2i=parenttot,
measure= "OR", data=dat, append=TRUE)
View(ex3)

#analysis with antibiotic as a covariate
res1 <- rma.mv(yi, vi, mods = ~ant-1, random = ~ factor(period) | factor(ID),
struct = "HAR", data = ex3)
summary(res1)

#analysis with animal as a covariate
res2 <- rma.mv(yi, vi, mods = ~factor(anim)-1 ,
random = ~ factor(period) | factor(ID), struct = "HAR", data = ex3)
summary(res2)

#analysis with bacteria as a covariate
FEM5<-rma.mv(yi, vi, data=ex3, mods= ~factor(bac)-1,
random = ~ factor(period) | factor(ID),
struct = "HAR")
summary(FEM5)

#analysis with year as a covariate
```

```
FEM5<-rma.mv(yi, vi, data=ex3, mods= ~Year,
random = ~ factor(period) | factor(ID),struct = "HAR")
summary(FEM5)

#analysis with  period as a covariate
FEM9<-rma.mv(yi, vi, data=ex3, mods= ~factor(period)-1,
random = ~factor(period) | factor(ID), struct = "HAR")
summary(FEM9)
```

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Richting: **Master of Statistics-Epidemiology & Public Health Methodology**
Jaar: **2018**

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