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QUANTIFICATION OF TOPICAL ANTIMICROBIALS IN DUTCH COMPANION ANIMAL VETERINARY CLINICS: DEVELOPING AND EVALUATING A QUANTIFICATION METHOD

Master's thesis

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Abstract

ABSTRACT

Background Topical antimicrobial (TAM) preparations are used extensively in human and companion animal medicine. Alterations in the bacterial flora of the treated area and emergence of resistant strains has been reported in humans, consequently to topical antimicrobial use (AMU). Resistant strains of staphylococci have also been isolated in companion animals and a reduction in the efficacy of these TAMs is of concern. Exposure to TAMs during their application by pet owners may be additionally hazardous to the owners due to toxic or adverse reactions to the drugs. Quantifying topical AMU in companion animal medicine could aid in the surveillance of antimicrobial resistance emergence and in determining the necessity of intervention strategies. Gaining more insight on topical AMU might also optimize dosing regimens, thus ensuring positive patient outcomes.

Objective The main objective of this study was to develop a quantification method, which could be used to quantify the use of TAMs in companion animal veterinary clinics and describe the topical AMU in 44 Dutch companion animal veterinary clinics.

Method This study consisted of four successive stages. In the first and second stage of the study, a literature review was conducted to identify possible quantification methods and their advantages and disadvantages were explored using a sample dataset. The third stage consisted of an experts' meeting, aiming to arrive at the final quantification method by consensus. Ultimately, the chosen quantification method was applied to a dataset containing monthly prescription data from 44 Dutch companion animal veterinary clinics and the seasonality, time trends and possible determinants of topical AMU were explored using statistical modelling.

Results The Defined Daily Dose for Animals (DDDA) quantification method was deemed as a suitable method to quantify the use of TAMs in companion animal veterinary clinics. A seasonal effect was found, suggesting that the total use of TAMs was highest in the months July-August and lowest in the months February-March. A significant decrease over time concerning total topical AMU was also observed. The proportion of dogs in the clinic appeared to affect the topical AMU. Clinics with a larger proportion of dogs had significantly higher total, first and second choice topical AMU. Likewise, ear and skin preparations were used significantly more in these clinics.

Conclusions This study displayed that the DDDA method is a suitable quantification method to quantify topical AMU. The analysis of retrospectively acquired data from 44 Dutch companion animal veterinary clinics in a three-year period (2012-2015) showed the existence of a seasonal effect and a decrease in topical AMU over time. The DDDA method should be better standardized for topical AMU in the future and the intervention effect on topical AMU should be further explored.

Plain Language Summary

Topical antimicrobial (TAM) medications are used extensively in humans and pets to treat various conditions affecting the skin, the eyes, the ears or the nose. It is important to note that the TAMs that are used in humans and in pets are largely identical. Typically, these medications are in the form of creams, ointments or drops and contain antimicrobial substances. This study focuses on the TAMs that contain antibiotic substances, which are used to combat bacterial infections. However, after applying these TAM medications in humans, bacteria that could resist the effects of the TAM that was applied were isolated. Similarly, resistant bacteria have been isolated from pets. This could lead to therapeutic failure, implying a negative health outcome for the patient. Worryingly, certain studies indicate that humans with resistant bacteria might pass them on to other humans through contact. Potentially, resistant bacteria or their resistance genes may also be transmitted between owners and their pets. Another important issue is that owners might come in contact with the TAM whilst applying it to their pets, which beside the possibility of promoting antimicrobial resistance (AMR), may cause health issues because of a reaction (e.g. a toxic or other type) to the medication. Therefore, concerns have been voiced regarding TAM use and its possible consequences regarding the health of both humans and animals, including its potential contribution to AMR emergence. If the amount of TAMs that are used was known, perhaps it would clarify their role in AMR emergence and aid in designing intervention strategies to mitigate this issue and preserve the efficacy of these medications.

This study's main goal was to identify and develop a method that could best measure the amount of TAMs that is used in companion animal veterinary clinics and subsequently, describe the topical antimicrobial use (AMU) that occurred in 44 Dutch companion animal veterinary clinics during a three-year period.

This study consisted of four successive stages. In the first and second stage of the study, methods that measure the amount of TAMs that are used in humans and animals were sought in the literature. The methods that were discovered were assessed based on their pros and cons and some were excluded. A panel of experts assessed the remaining methods during a meeting at the third stage, which aimed at selecting one method by consensus. In the fourth and final stage of the study, the selected method was applied to the 44 Dutch companion animal veterinary clinic dataset in order to explore whether certain characteristics of the clinics might influence the topical AMU. Additionally, to explore whether in certain months a higher or a lower topical AMU was observed and lastly, whether during that three-year period the topical AMU changed (e.g. increased, decreased) or remained the same.

The selected method was the Defined Daily Dose for Animals (DDDA). Using this method, it was discovered that the 44 Dutch companion animal clinics used the most TAMs in the months July-August and the least in February-March. Additionally, during that three-year period it

appeared that the amount of TAMs that were used by these clinics decreased. Lastly, clinics that had a higher amount of dogs as patients, appeared to use more TAMs.

This study identified and developed a method to measure the amount of TAMs that are used in companion animal clinics. A decrease in total topical AMU over a three-year period and a seasonal pattern was discovered using data from 44 Dutch companion animal clinics. In the future, estimating the impact of an intervention strategy on topical AMU in the same dataset would give more information on the effectiveness of such mitigation strategies.

Introduction

Historically, the main cause of death for humans was infection caused by microorganisms, such as bacteria, viruses, fungi and parasites (1,2). The discovery of antibiotics, first of arsphenamine in 1909 by Paul Ehrlich and subsequently, of penicillin by Alexander Fleming in 1928 marked a new era in medicine and importantly, in the treatment of bacterial infectious diseases (3–5). Likewise, effective antibiotics have been integral in treating infectious diseases in animals and establishing animal welfare conditions (6,7). Yet, the successful therapeutic outcomes of using antibiotics in clinical practice were soon followed by the emergence of antibiotic resistant bacterial strains (8). Antimicrobial resistance (from now on AMR) constitutes the capability of microorganisms, such as bacteria, to become resistant to the effects of antimicrobial medicines to which they were previously susceptible (9). AMR is a major threat to global health as it threatens the effectiveness of the medications used to prevent and treat infectious diseases (10). Without effective antibiotics, both standard and major surgical operations as well as chemotherapy would be nearly impossible to be performed as antibiotics are used prophylactically in these instances (7,11). Hence, previously curable infectious diseases may result in infections, deaths and substantial economic losses to societies because of AMR (10,12). Importantly, by 2050, estimations demonstrate that 10 million deaths globally will be attributable to AMR each year which will be associated with USD 100 trillion in economic losses (13).

The two principal drivers of AMR are considered to be antimicrobial use-appropriate and inappropriate-and the transmission of antimicrobial (in this thesis with this term we refer to antibiotics) resistant bacteria and genes between humans, animals and the environment (14–16). Bacteria can have the innate ability to resist the effects of an antibiotic as a consequence of the bacteria's structural and functional characteristics (4,17–19). However, bacteria can also acquire AMR because of a genetic mutation or due to the acquisition of new genetic material from an exogenous source (4,17,19). Selection pressure from antimicrobial use (from now on AMU) appears to provide a competitive advantage for resistant bacterial strains, which can outgrow non-resistant bacterial strains in the presence of an antibiotic (4,18,20). Significantly, through mechanisms such as horizontal gene transfer, this resistance may be transferred from one bacterium to another within and between bacterium and animal species (15,17,21). This indicates that the use of antimicrobials in one sector can potentially have an impact on the other

sectors (21–23). Notably, the antimicrobials that are used in both the human and animal sector to combat infectious diseases are largely identical while certain pathogens may also be shared (7,12,22,24). Consequently, AMR is a complex issue that needs to be approached in a holistic manner. Indeed, efforts are currently concentrated in using the One Health approach. One pillar is the development and implementation of antimicrobial stewardship programmes (ASPs), to control and prevent the progression of AMR globally (9,10,15). ASPs are defined as a coherent set of actions which promote using antimicrobials responsibly (25). Primarily, the aim of the ASPs is to ensure positive patient outcomes whilst reducing the inadvertent effects of AMU, which also encompass the development of AMR (26).

The role of antimicrobial use in antimicrobial resistance

The World Health Organization (WHO) issued in 2015 the "Global Action Plan on Antimicrobial Resistance" in which it includes in its objectives the importance of optimizing the use of antimicrobials in human and animal health alike (10). Any antimicrobial use can select for antibiotic-resistant bacterial strains, thus driving the emergence and dissemination of resistance (14). Several studies have demonstrated significant correlations between antimicrobial consumption and the prevalence of resistant strains within populations at a national or regional level (27–32). Likewise, at the individual level, use of antimicrobial medicines has been shown to alter the normal microbiota, specifically giving rise to resistant bacterial populations which may persist long-term (33–36). This phenomenon is further accentuated when the use of antimicrobials is inappropriate (4,10,12). Overuse of antimicrobials accelerates the emergence of resistance by means of natural selection thereby inhibiting susceptible microorganisms and selecting those that are resistant (20). Despite this, antimicrobial consumption in humans worldwide appears to be on the rise as Van Boeckel et al. report a 36% increase in consumption between 2000 and 2010 (37). The observed surge in antimicrobial consumption may possibly be attributed to inappropriate prescriptions, self-medication and over the counter sales of antibiotics as well as prophylactic use of antimicrobials (37). An additional contributing factor in the emergence of AMR is the misuse of antimicrobials (12,38,39). Particularly, antimicrobials at subinhibitory concentrations can possibly hasten the evolution of resistance via increasing genetic alterations in bacteria, such as mutagenesis and recombination (38–40).

Aside from the human sector, large amounts of antimicrobials are being used in the agricultural sector (23). Despite several countries having banned their use as growth promoters (41), other countries still use antibiotics in this manner as well as for prophylaxis (12,23,42,43). This may lead to subinhibitory doses being administered, thus potentially further promoting AMR emergence. Strikingly, it has been estimated that in most countries, more than 50% of the medically important antimicrobials are being used in livestock (23). Although zoonotic transmission of resistant food-borne bacterial strains is rather complex to decipher, diseases caused by such microorganisms in humans have been identified (42,44). Remarkably, the emergence of resistance in foodborne zoonotic *Salmonella* and *Campylobacter* bacteria has been associated with AMU in food-producing animals, thereby emphasizing the public health risk that imprudent use of antimicrobials in this sector may cause (42,44,45).

Companion animals: antimicrobial use and antimicrobial resistance

Over recent years, companion animal populations have experienced an increase and so has the demand for higher health standards for them as owners tend to live in closer contact with their pets (46). Inevitably, use of antimicrobials in companion animal veterinary medicine has also risen with some of the antimicrobials that are being used, belonging to the category of critically important to human medicine (46–48). A strong selective pressure is induced by the increased consumption of these antimicrobials, thus possibly promoting the emergence of not only resistant strains but also multidrug-resistant (MDR) ones (48). Currently, a substantial number of MDR pathogens have been identified in companion animals, including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), MDR *Acinetobacter baumanni* but also Extended Spectrum Beta-Lactamase (ESBL), carbapenemase and AmpC-type β-lactamases (AmpC) producing Enterobacteriaceae, raising serious concerns regarding both animal and public health (46,49–52). MRSA is one of the most significant bacteria which cause hospital and community-acquired infections in humans and most of MRSA that have been isolated from companion animal patients, have been identical to human hospital-acquired MRSA lineages (52). Although MRSA prevalence in dogs and cats is rather low, certain studies have shown the possibility of MRSA being passed from pets to owners with the potential of zoonotic infections occurring (53–56). Importantly, dogs and cats may acquire MRSA from humans (57,58) and consequently, may serve as a reservoir of human MRSA (59). A more substantial health issue for companion animals themselves constitutes the emergence of MRSP which is able to cause a variety of infections, such as skin and ear infections as well as peritonitis and septicaemia, in canines and felines (50). Significantly, MRSP is resistant to most oral and parenteral antimicrobials approved for veterinary use (50). Notably, veterinarians who are in contact with infected with MRSP pets and the owners of infected pets, seem to be at a higher risk of being MRSP positive (50).

Topical antimicrobials

Topical antimicrobials (TAMs) are being used extensively in human medicine as dermatologists often use them for treating bacterial infections of the skin, the nose or pre- and post-operatively to surgical wound sites (60–64). The main advantages of TAMs, compared to systemically administered antimicrobials, is the fact that they reach a higher concentration at the site that they are applied to and that they generally have less systemic side effects (64–66). However, it has been established that use of TAMs may alter the bacterial flora of the treated area and particularly, in chronic conditions, in which long-lasting treatments are indicated, selection of resistant bacteria has been observed (67,68). Worryingly, certain studies have indicated the possibility of interpersonal spread of these resistant strains (67,68). Another troublesome observation is the emergence of two types of mupirocin (pseudomonic acid A) resistance concerning *Staphylococcus aureus*; high-level and low-level mupirocin resistance (69,70). Mupirocin is indicated for use in the elimination of nasal carriage of staphylococci, including MRSA, thus retaining the efficacy of this TAM is highly important (71). Use-and particularly, the increased use- of mupirocin may contribute to the emergence of mupirocin resistant strains (70,72,73). Indicatively, the widespread and unrestricted use of mupirocin in

New Zealand was associated with a rise in mupirocin resistance among *S. aureus* isolates from approximately zero in the early 1990s to 28% in 1999 (74). Similar observations were also made in Canada concerning mupirocin resistance (75). Fusidic acid resistance in staphylococcal strains has also been described (76). As with mupirocin, its widespread use in New Zealand has been associated with an increase in resistance against it and a potential decrease in its efficacy (77–79).

In companion animal veterinary medicine, topical antimicrobials are also used in several instances either in combination with a systemic antibiotic or exclusively (80–82). The majority of topical medicines used in companion animals consists of formulations such as ear or eye drops, typically used in dogs and cats (81). For skin applications, topical antibacterial preparations usually consist of ointments, gels and creams (81,83,84). Concerns in human medicine have been voiced with regard to the possibility that these topical antimicrobial agents might experience a reduction in efficacy, for example concerning fusidic acid (85,86). One major concern regarding the use of TAMs is their potential contribution to the emergence of resistant and MDR strains (87,88). Importantly, mupirocin resistant staphylococci have occasionally been isolated from companion animals, including high level mupirocin resistant strains, despite mupirocin not being registered for use in animals in most countries (87–90). It is important to mention that the close contact of owners with their pets may potentially contribute to the spread of resistant strains, including mupirocin resistant ones (87). ARGs from human *S. aureus* isolates to canine *S. pseudintermedius* may also potentially be transmitted and vice versa (87,88). Consequently, imprudent AMU could possibly promote the emergence of high level mupirocin resistance in companion animals in the future, which poses a public health risk (87). The application of TAMs in the form of ointments, creams and gels could additionally be problematic in hairy skin and may impact the therapeutic outcome (84,91). Additionally, compliance problems may lead to underdosing, missed doses and stopping the treatment early, thus potentially promoting the emergence of resistant strains (40,92). Importantly, owners may be exposed to the topical antimicrobial when they apply it to their pets, which may be hazardous not only because of the possibility of AMR emergence but also because of health implications due to toxicity or because of other adverse reactions to the drug.

Currently, both in human and companion animal veterinary medicine, specific breakpoints for topical agents concerning minimum inhibitory concentrations (MICs) are lacking (66). In light of the scarcity of research concerning use of TAMs, determining the effective dose, the effective interval and the safety of an alteration in the dosing regimen of TAMs is difficult to achieve (66). Gaining more insight on the use of TAMs would not only aid in elucidating its relationship to AMR emergence but also potentially optimizing dosing regimens, thus minimizing other inadvertent effects and ensuring positive therapeutic outcomes.

Study Objectives

To date, standardized quantitative data on systemic AMU in companion animals are scant and even scarcer are the respective data on topical AMU (93). The first objective of this study was to develop and evaluate a robust method to quantify the use of topical antimicrobials. The second objective of this study was to quantify and describe the topical AMU in 44 Dutch companion animal veterinary clinics over a three-year period. To that end, four research questions were formulated:

1. Which methods exist for the quantification of AMU in humans and animals for systemic and topical antimicrobials?
2. What are the advantages and disadvantages of the quantification methods that were identified?
3. Which quantification method is considered the best for quantifying topical AMU?
4. Can time trends, seasonality and determinants in the topical AMU be explored over a three-year period in 44 Dutch companion animal veterinary clinics using the selected method?

Materials and Methods

In Figure 1, the successive stages of the study design are outlined.

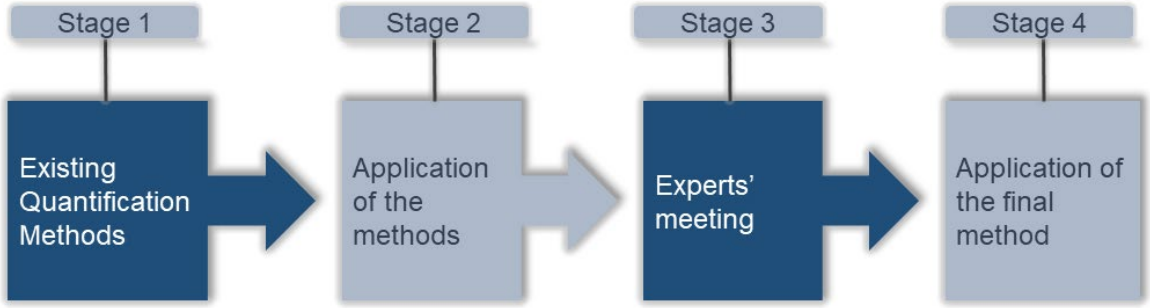


Figure 1: Successive stages of the study design.

The datasets that were used in stages two and four, were obtained from the former Antimicrobial Stewardship and Pets (ASAP) project which mainly focused on quantifying systemic AMU (94,95). In particular, the ASAP project was designed as a prospective, stepped-wedge intervention study in which an antimicrobial stewardship programme intervention took place from March 2016 until March 2018. Additionally, pre-intervention data were acquired retrospectively from 2012 until 2015 (94). Consequently, the datasets include topical antibiotic prescription data and clinic animal population data derived from 44 Dutch companion animal veterinary clinics in the period from July 2012 until June 2018. This data were monthly prescription data with only six clinics providing daily prescription information. For this study only data from 2012 until 2015 (pre-intervention) were used.

Stage 1: Existing Quantification Methods

In the first stage of the study, a literature review was conducted with the aim to identify the already existing quantification methods in both human and veterinary medicine, regardless of route of administration. Subsequently, the search was narrowed down to quantification methods in veterinary medicine and, following that, in companion animal veterinary medicine. Methods that quantify topically administered medicines were also sought in both the human and animal sector. The quantification methods that were identified were subsequently assessed by distinguishing the advantages and disadvantages of each method. Based on this assessment, the methods were ranked as "suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics" or "not suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics".

Data collection

The online scientific search engine PubMed was used to discover pertinent literature on the quantification methods that are used in human and animal medicine. Initially, the terms "antimicrobial consumption quantification" and "antimicrobial stewardship metrics" were entered into the engine, resulting in 403 and 319 hits, respectively, which included quantification metrics used in human medicine. Subsequently, the search was specified to include only the animal sector, thus the term "quantification of antimicrobial consumption in animals" was entered, which resulted in 124 hits. Lastly, in order to discover the quantification methods that are in existence pertaining companion animal veterinary medicine, the term "companion animals" was added, resulting in 29 hits. Two literature review papers (96,97) were also utilized to discover additional papers regarding quantification methods in food-producing animals and companion animals. Furthermore, the official site of the WHO and the European Medicine Agency (EMA) were navigated and the proposed methodology concerning antimicrobial consumption surveillance in humans and in animals was retrieved (98,99). Concerning topically administered medicines, the 2020 NethMap-Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (MARAN) report (100), which informs on AMR and AMU in the Netherlands in both humans and animals, was consulted. Similarly, the G-Standaard website (101), which contains information and relevant data on healthcare products-including units of measurements for medicines-in the Netherlands, was searched.

Data analysis

From the entirety of the papers and reports that were identified during the data collection phase, 64 were used to ascertain possible suitable quantification methods for the quantification of topical antimicrobials in companion animal veterinary clinics. This was performed by critically assessing the advantages and disadvantages of each quantification method in terms of feasibility, complexity and the nature of the expected outcomes. The assessment was carried out by the Quantification of Topical Antimicrobials (QUANTA) group (Appendix I). Following that, the quantification methods were ranked accordingly and solely those ranked as

"suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics" were used in the second stage of the study.

Stage 2: Application of the methods

The methods that were ranked as "suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics" in the previous stage of the study, were applied in a sample dataset in the second stage of the study. Due to the fact that the size of the sample dataset was small, the results of this analysis could not be generalized and served only as an example to showcase the nature of the outcomes of each method and potential differences between them. Subsequently, the results were visualized with the aim to better distinguish the differences in the nature of the outcomes produced by the respective methods. Concurrently, a video presentation containing a detailed tutorial explaining the calculation process of each quantification method was recorded.

Data collection

The sample dataset that was used in this stage was a subset of the full dataset, which derived from the former ASAP project (94,95). Particularly, it contained pre-intervention topical antibiotic prescription data and animal population data from six-of the initial 44-Dutch companion animal veterinary clinics and spanned over a period of three years, from July 2012 until June 2015. Importantly, this sample dataset was selected as it contained the additional information of which and how many topical antibiotic packages were dispensed on one specific day to one specific animal patient. In addition to that, the species in which that animal patient belonged to was also known. This enabled displaying more differences and more details between the selected methods.

Data analysis

The sample dataset was provided as a Microsoft Excel spreadsheet, which was subsequently imported in the Integrated Development Environment "RStudio" and analysed using the programming language R. The visualization of the outcomes was also conducted using the programming language R in "RStudio" with the "ggplot2" package. In order to assess the topical antimicrobial use that occurred in each clinic, the annual average of the topical antimicrobial use per individual animal and with regard to each clinic was measured separately for each quantification method. Using this analyses, the following could be determined: (i) which clinic used the most TAMs on average in a year and per individual animal, (ii) which animal species was exposed to the most topical AMU, (iii) what topical antimicrobial products were used the most and (iv) which antibiotic category (first, second or third choice antibiotics) were used the most on average in a year and per individual animal. Details on the calculation process of the methods were presented in a tutorial video that was created, as well as recorded, with Microsoft PowerPoint.

Stage 3: Experts' meeting

The third stage of the study consisted of an experts' meeting with the aim to evaluate and rank the identified quantification methods and arrive at a final method based on consensus of the participants. Preparatory presentations were shared with the participants to introduce them to the concepts and quantification methods that were considered before and during the meeting. A survey was completed by the experts during the meeting and an open discussion was conducted afterwards.

Process & analysis

An experts' meeting was held on the 23rd of June 2021 with five participants that were contacted and invited to an online Microsoft Teams meeting via e-mail. The participants consisted of two experts in veterinary dermatology, one animal shelter veterinarian, one companion animal veterinarian in practice and one expert in veterinary epidemiology and antimicrobial consumption. In preparation of this meeting, the Microsoft PowerPoint tutorial video presentation that was recorded in stage two of the study was sent to the experts one week before the meeting along with a PDF document containing the slides and the information conveyed in the tutorial video, in written format. Moreover, a survey was prepared using the online interactive presentation software "Mentimeter". This survey contained four questions, which would ask the experts to rank the methods accordingly at a specific point during the meeting. On the day of the meeting, a presentation was, firstly, performed detailing the objectives of the project, the quantification methods that were applied and the outcomes of the analyses that was performed on the second stage of the study, which concerned the sample dataset. Additionally, the differences between the methods were showcased by use of examples and ultimately, the advantages and disadvantages of each method were outlined. The experts were informed that questions could be posed during the presentation as well as after its end. Subsequently, the survey was implemented using Mentimeter. The results of each question were displayed after the entirety of the four questions had been ranked by all the experts. It is important to note that voting anonymity was maintained during and after the ranking process had been completed. Thereafter, a discussion session was initiated to agree on a final quantification method for topical antimicrobials in companion animal veterinary clinics by consensus.

Stage 4: Application of the final method

In the fourth and final stage of the study, the quantification method that was selected by consensus during the experts' meeting was applied to the full dataset of the 44 Dutch companion animal veterinary clinics. Subsequently, the statistical analysis of the outcomes was carried out in order to explore the time trends, the seasonality and potential determinants concerning topical AMU, with regards to the pre-intervention period.

Data collection & analysis

The dataset consisted of the monthly pre-intervention and post-intervention prescription data from the former ASAP project with regard to topical antibiotics, which derived from the 44

Dutch companion animal veterinary clinics. One period amounted to one year and spanned from July of the previous year to June of the next. The mean total topical AMU was estimated for each period of the study based on the data from the participating clinics. Overall, the dataset included data that ranged from July 2012 until June 2018 per month, however, the statistical analyses was limited to the pre-intervention period which spanned from July 2012 until June 2015.

The characteristics of each clinic were included in the dataset from the former ASAP project, which were initially collected through a questionnaire in the respective study (94). Subsequently, these characteristics were assessed as possible determinants using a multivariable regression model with log-transformed topical AMU data. Topical AMU data were categorized in two different ways: total, 1st, 2nd , 3rd choice topical AMU and AMU with regard to ear, eye, skin, nasal products and topical products that their application was unknown regarding companion animals. During the analysis it appeared that the number of veterinarians was highly correlated (Pearson's $r = 0.75$) with the total number of dogs, cats and rabbits, therefore it was excluded from subsequent analyses. Similarly, because the number of cats was highly correlated (Pearson's $r = 0.75$) with the proportion of dogs, it was also excluded from subsequent analyses to reduce collinearity between variables. The statistical significant P-values were equal or smaller than 0.05, whereas P-values that ranged between 0.05 and 0.10 were viewed as almost statistically significant, demonstrating a trend or tendency.

Seasonal effects were modelled with a combination of two pairs of harmonic (sine/cosine) functions. Time trends were modelled using natural regression splines with a single interior knot placed at the median time point (January 2014). The statistical model included clinic-specific intercepts and trend coefficients for random effects while an auto-regressive (AR1) correlation structure for the residuals was also used. These were implemented in order to permit heterogeneity between clinics, concerning time and seasonal trends, while also to account for the autocorrelation of observations within a clinic over time. To permit for the interpretation of the fixed effects as the ratio of geometric means (GMR) of estimated topical AMU, the natural spline bases were centred at the interior knot placement and interpreted at this specific point in time (month of May).

The analyses of the dataset and the visualization of the outcomes were conducted by using the programming language R in "RStudio" and particularly, the package "nlme" (version 3.1) was used for the statistical analyses. Additionally, the final method's advantages and disadvantages were further assessed.

Results

Stage 1: Existing Quantification Methods

In total, seven quantification methods that were identified from the literature were considered as possible metrics for quantifying the use of topical antimicrobials in companion animal

veterinary clinics (96,98,99,101–106). From these, four quantification methods were deemed as “suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics” based on a critical assessment of the advantages and disadvantages of all the possible metrics. In Table 1, the advantages and disadvantages of the seven considered metrics are delineated. The four quantification methods that were considered as “suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics” were the Total grams of active substances (96,103), the Fingertip Unit/Droplet Dose (FTU/DD) (101,107), the Number of packages sold (96,103) and the Defined Course Dose (DCD or Number of treatment courses) (96,99,103).

Metric	Advantages	Disadvantages
Total grams of active substances	▪ Not reliant on dosages, days of treatment or the weight of the animals ▪ Available input data ▪ Method is known	▪ Possible discrepancies due to not accounting for differences in dosages ▪ Product volumes influence the results ▪ Calculation is tedious
Fingertip Unit/Droplet Dose (FTU/DD)	▪ Easy to calculate ▪ Not reliant on dosages, days of treatment or the weight of the animals ▪ Available input data	▪ Method is not known ▪ Product volumes influence the results ▪ Does not account for potencies, concentrations of active substances and dosages
Number of packages sold	▪ Easy to calculate ▪ Not reliant on dosages, days of treatment or the weight of the animals ▪ Available input data ▪ Method is known	▪ Possible discrepancies due to differences in available product volumes in different clinics
Defined Course Dose (DCD or Number of treatment courses)	▪ Easy to calculate ▪ Not reliant on dosages, days of treatment or the weight of the animals ▪ Available input data ▪ Method is known	▪ An assumption is necessary when defining the treatment course
Days of therapy (DOT)	▪ Easy to calculate ▪ Not reliant on dosages, the weight of the animals ▪ Method is known	▪ Not available input data (days of treatment and patient-specific data)
Defined Daily Dose for intramammary products	▪ Not reliant on the weight of the animals ▪ Method is known	▪ Species-specific calculation (mainly used in cows), thus possibility of discrepancies ▪ Only applicable for intramammary products that are used as TAMs in companion animals ▪ Assumptions are necessary ▪ Not available input data (dosage for companion animals) ▪ Calculation is more complex
Defined Daily Dose Animal (DDDA)	▪ Method is known ▪ A more standardized method for AMU measurement ▪ Can be used for benchmarking	▪ Lack of available input data (daily dose for TAMs, patient-specific data) ▪ Assumptions in estimating daily dose and duration of therapy ▪ Is reliant on the weight of the animals

Table 1: Advantages and disadvantages of the seven considered quantification methods.

Measurement of antimicrobial consumption

In order to quantify the use of antimicrobials, certain so-called 'indicators' of AMU are utilized (103,108). As Collineau et al. mention, the definition of these 'indicators' is "the number of 'technical' units of measurements (i.e. amount of antimicrobials) consumed and normalized by the population at risk of being treated in a defined period" (103). Importantly, the EMA further clarifies that the word 'technical' is used to designate that the units of measurements are not considered as traditional units of measurements, such as kilograms (which is used to measure the physical quantity of weight, for instance), but contrarily they represent theoretical reference values for expressing the consumption of antimicrobial agents (103,109). Currently, the methods that are implemented for reporting antimicrobial consumption, in both human and animal medicine, are expressed as a rate (102,103,108). Specifically, the numerator is the metric that measures consumption-the amount of antimicrobial usage- and the denominator is the measurement of the population at risk of being treated (102,103,108). In particular, the numerator is expressed with a unit that is termed Unit of Measurement (UM) (108). There are three categories of UMs; mass-based, dose-based and count-based (108). In mass-based UMs, the numerator is expressed in milligrams, kilograms or tons of the active substance whereas in dose-based UMs, the numerator is expressed in number of doses (108). Concerning count-based UMs, the numerator may express the number of treatment courses or treatment days (108).

Calculation of the four quantification methods

Of the four quantification methods that were considered, two had a mass-based UM (Total grams of active substances, FTU/DD) and two had a count-based UM (Number of packages sold, DCD). In Table 2, the breakdown of the formulas of the four quantification methods are displayed along with their UMs.

1. Total grams of active substances

The "Total grams of active substances" method is calculated as a fraction in which the numerator is equal to the number of packages of the respective TAM that were sold on a defined time period, multiplied by the package volume and its strength. However, because the outcome of this multiplication is in milligrams, by dividing it by 1000 it is converted into grams.

$$\text{Numerator} = \frac{Number\ of\ packages\ sold \times package\ volume \times strength}{1000}$$

The denominator of the fraction is the animal population that attended the clinic on the defined time period. In this study, the animal population consisted of the number of dogs, cats and rabbits that attended the clinic.

$$\text{Denominator} = \text{Animal population} = Number\ of\ dogs + number\ of\ cats + number\ of\ rabbits$$

Thus, the full formula of the "Total grams of active substances" is the following:

$$\text{Total grams of active substances} = \frac{\frac{Number\ of\ packages\ sold \times package\ volume \times strength}{1000}}{Animal\ population}$$

Based on the formula of this quantification method, it is understandable that the "Total grams of active substances" method is mainly influenced by two parameters; the volume of the TAM package and the concentration of the active substances (e.g. the strength of the product). Particularly, it may be the case that higher volume packages will result in a higher "Total grams of active substances" compared to lower volume products. Nevertheless, it may also be the case that a low volume product that has a high concentration of active substances may result in a larger "Total grams of active substances" than a product that has a higher volume but a lower concentration of active substances. It is important to note that with this quantification method, the outcome is expressed in mass of active substances (thus, grams of active substances).

2. Fingertip Unit/Droplet Dose (FTU/DD)

The "Fingertip Unit/Droplet Dose (FTU/DD)" method is a quantification method that was adjusted to the purposes of this study. Initially, this method was developed to quantify the use of corticosteroid creams and ointments in human medicine, thus being named as "Fingertip Unit" (107). The amount of a cream or an ointment that is applied to the first phalanx of the index finger of an adult human was defined as one fingertip unit, which is equal to 0.5 grams of the cream or ointment (107). Besides creams and ointments, which are used in skin conditions in companion animal veterinary medicine, drops and solutions are also used extensively. Consequently, this quantification method was adjusted by the QUANTA group to additionally quantify these TAM products. Subsequently, one droplet dose was defined as 0.5 mL, which corresponds to 10 drops of a solution. Thus, the name of this quantification method was finalized as "Fingertip Unit/Droplet Dose (FTU/DD)".

The "FTU/DD" method is calculated as a fraction in which the numerator is equal to the number of packages that were sold on a defined time period multiplied by the volume of the TAM package. The outcome of this multiplication is then divided by 0.5. The reason for this division is that one Fingertip Unit or Droplet Dose is assumed to be equal to 0.5 grams or mL, respectively.

$$\text{Numerator} = \frac{Number\ of\ packages\ sold \times package\ volume}{0.5}$$

The denominator of the fraction is the animal population that attended the clinic on the defined time period, as with the previous method.

$$\text{Denominator} = \text{Animal population} = Number\ of\ dogs + number\ of\ cats + number\ of\ rabbits$$

Thus, the full formula of the "Fingertip Unit/Droplet Dose (FTU/DD)" is the following:

$$\text{FTU/DD} = \frac{\frac{Number\ of\ packages\ sold \times package\ volume}{0.5}}{Animal\ population}$$

Based on the formula of the "FTU/DD" quantification method, the only variable that influences the result is the volume of the TAM package. Thus, with high volume products, the FTU/DD will also be high and with lower volume products, the FTU/DD will be low. Consequently, this quantification method is analogous to the volume of the products. The outcome of the FTU/DD quantification method is expressed in mass per fingertip unit or droplet dose, thus grams per fingertip unit or mL per droplet dose. More specifically, each TAM product can be viewed as a collection of either fingertip units or droplet doses, depending on the formulation of the product, which is based on its volume.

3. Number of packages sold

The "Number of packages sold" method is also calculated as a fraction in which the numerator is the number of packages that were dispensed on a defined time period and the denominator is the animal population, as described in the previous methods.

$$\text{Numerator} = Number\ of\ packages\ sold$$

$$\text{Denominator} = \text{Animal population} = Number\ of\ dogs + number\ of\ cats + number\ of\ rabbits$$

The full formula of the "Number of packages sold" quantification method is as follows:

$$\text{Number of packages sold} = \frac{Number\ of\ packages\ sold}{Animal\ population}$$

The outcome of the "Number of packages sold" method is expressed in the total number of packages of a TAM product that was dispensed in a defined period of time.

4. Defined Course Dose (DCD or Number of treatment courses)

The "Defined Course Dose (DCD or Number of treatment courses)" method is calculated similarly to the "Number of packages sold" method with the only difference being that in the numerator, instead of the number of packages that were sold on a defined time period, the number of treatment courses are used. To better clarify this, one treatment course is defined as the number of packaged that were dispensed on one specific day and to one specific animal-patient. Thus, for this method to be calculated, daily prescription data are necessary. The reasoning behind this method is that the number of packages that were dispensed on the specific day to the specific animal-patient are deemed as necessary in order to complete the treatment of this animal-patient. The denominator of this method is the same as with the previously described methods.

$$\text{Numerator} = Number\ of\ packages\ sold$$

Denominator = Animal population = $Number\ of\ dogs + number\ of\ cats + number\ of\ rabbits$

The full formula of the "DCD (or Number of treatment courses)" quantification method is as follows:

$$\text{DCD} = \frac{Number\ of\ treatment\ courses}{Animal\ population}$$

The "DCD (or Number of treatment courses" method looks at packages that were dispensed. The only difference from the "Number of packages sold" method is that when the amount of packages that were dispensed on a specific day and to a specific animal-patient is known, then that is termed as one treatment course, which is precisely what the "DCD" method measures.

Metric	Numerator	Denominator	Full formula	Unit of measurement (UM)
Total grams of active substances	$\frac{Number\ of\ packages\ sold\ \times\ package\ volume\ \times strength}{1000}$	Animal population	$\frac{\frac{Number\ of\ packages\ sold\ \times\ package\ volume\ \times strength}{1000}}{Animal\ population}$	Mass-based
Fingertip Unit/Droplet Dose (FTU/DD)	$\frac{Number\ of\ packages\ sold\ \times\ package\ volume}{0.5}$	Animal population	$\frac{\frac{Number\ of\ packages\ sold\ \times\ package\ volume}{0.5}}{Animal\ population}$	Mass-based
Number of packages sold	$Number\ of\ packages\ sold$	Animal population	$\frac{Number\ of\ packages\ sold}{Animal\ population}$	Count-based
Defined Course Dose (DCD or Number of treatment courses)	$Number\ of\ treatment\ courses$	Animal population	$\frac{Number\ of\ treatment\ courses}{Animal\ population}$	Count-based

Table 2: Formula breakdown of the four methods.

Data analysis

With regard to this study and the available data, the four methods that were selected had nearly the same advantages. In particular, the majority of them are easy to calculate-with the exception of the "Total grams of active substances" method, which is more tedious to calculate comparably. In addition to that, the entirety of the four methods do not require the dosages and the days of treatment in order to be estimated, which is advantageous seeing that concerning TAMs, this information is not available for all products based on the Summary of Product Characteristics (SPC) nor based on the dataset that was used. The weight of the animals is additionally not required for the calculation of these methods, which is an advantage since the animal population of the clinic is mixed, with regard to the species, while also the exact weight of each animal was not indicated in the dataset. For all four quantification methods, the input data that were required to calculate each method were included in the dataset, thus they could all be estimated. A final advantage that the majority of these methods share is that they are familiar methods to veterinarians with the exception of the "Fingertip Unit" method, which has only been proposed in human medicine for quantifying corticosteroid creams (107). Nevertheless, certain disadvantages were also identified. When estimating the "DCD (or Number of treatment courses)" method, an assumption is necessary to define the treatment course. Specifically, the number of packages of a TAM that were dispensed on a specific day and to a specific animal-patient is termed as 'one treatment course'. Consequently, the number of TAM packages that were dispensed on a particular day and to a specific animal-patient are necessary input data when calculating the DCD and this information was only available for six of the initial 44 Dutch companion animal veterinary clinics in this study. Thus, this method could only be applied to these clinics. The "Number of packages sold" method does not need such an assumption to be estimated as it is calculated by measuring the TAM packages that were dispensed from a clinic on a defined time period. However, there is a possibility of over- or under-estimating the AMU of a clinic because different clinics might be using the same products but in different volumes that may be available. For instance, it is understandable that if a clinic dispensed one skin cream of 60 mL in a specific time period and another clinic dispensed one package of 120 mL of the same skin cream in the same time period, these two clinics would be considered as having used the same whereas in reality, the second clinic has used more. The "Total grams of active substances" method is comparably more tedious in its calculation while also discrepancies are possible because it does not account for the differences in dosages. In addition to that, the volume of the products may influence the results. The disadvantage of the "FTU/DD" method lies in the fact that it is highly influenced by the volume of the products, as it does not take into account the differences in dosages, potencies or concentration of the active substances. Additionally, this method is not familiar to veterinarians.

The three quantification methods that were considered initially but subsequently, were not deemed as "suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics" were the "Days of therapy (DOT) (102), "Defined Daily Dose for intramammary products" (106) and the "Defined Daily Dose for Animals (DDDA)" (104). The

main reason for excluding these metrics was the lack of the necessary input data to calculate them. Particularly, for the DOT method patient-specific data and the days of treatment with a specific TAM are necessary, which in this study were not available. Concerning the Defined Daily Dose for intramammary products, the daily dose could not be ascertained for use in companion animals since these products are mainly used in livestock, thus assumptions would be necessary and the possibility of discrepancies would be substantial. With regard to the DDDA method, this has been applied to companion animals but for systemic antimicrobials (104). In order to calculate the DDDA, the total animal mass in kilogram that can be treated for one day with the amount of antimicrobials prescribed and the total weight in kilogram of the clinic animal population at risk to be treated are needed. Concerning TAM products, in this study, the information on the daily dose was not available for all of the products based on the SPC.

Stage 2: Application of the methods

Of the six clinics that were analyzed with the four quantification methods, two clinics were deemed as the ones to have used the most topical antimicrobials in the observed time period, when using different quantification methods. Specifically, with the "Total grams of active substances" and the "FTU/DD" methods, clinic number three came out as having used the most TAMs on average in a year whereas with the "Number of packages sold" and the "DCD" method, clinic number two came out as the one to have used the most TAMs on average in a year.

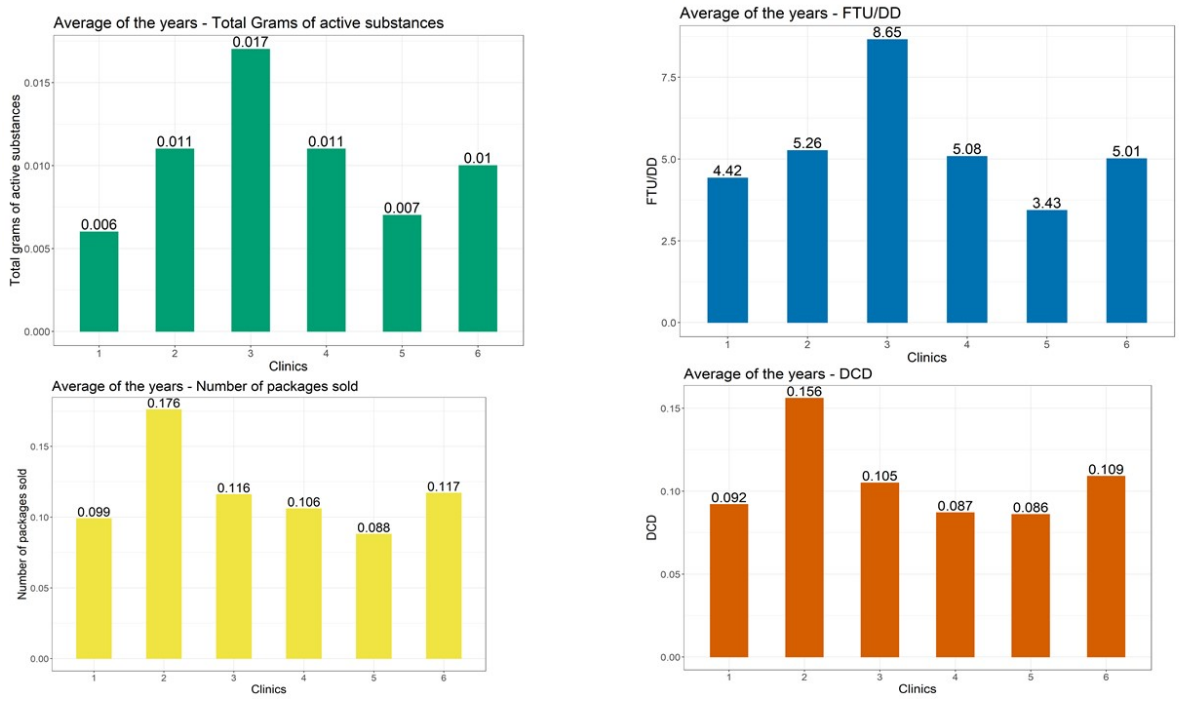


Figure 2: Outcome of the four quantification methods concerning the amount of TAMs used by the six clinics on average in a year to an individual animal.

In Figure 2, the amount of TAMs that were used on average in a year to an individual animal by the six clinics after the application of each of the four quantification methods is presented. More accurately, based on Figure 2, 17 mg of mass of active substances are received by an individual companion animal on average in a year from clinic number three, when the "Total grams of active substances" method was applied. Similarly, when the "FTU/DD" method was applied, approximately nine FTU/DD are applied on average in a year to a companion animal from clinic number three. Alternatively, with the methods "Number of packages sold" and "DCD", clinic number two dispenses on average approximately one package every five years to a companion animal. Thus, with the application of different quantification methods to the dataset, different outcomes were observed.

The species that was exposed to the most TAMs on average in a year in all the six clinics and with all the four quantification methods were dogs. Then, second to have been exposed to the most TAMs were cats and the species that was exposed to the least TAMs were rabbits. However, depending on the quantification method and the category of antibiotics the outcomes were slightly different. In Figure 3, the outcomes of the analysis concerning first choice topical antibiotics per species, for each clinic and quantification method are visualized. In clinic number five, with regard to the methods "Total grams of active substances" and "FTU/DD", cats have been exposed to approximately the same amount of TAMs as dogs. By contrast, in the "Number of packages sold" and "DCD" methods, in clinic number five cats have been exposed to more TAMs than dogs. In addition

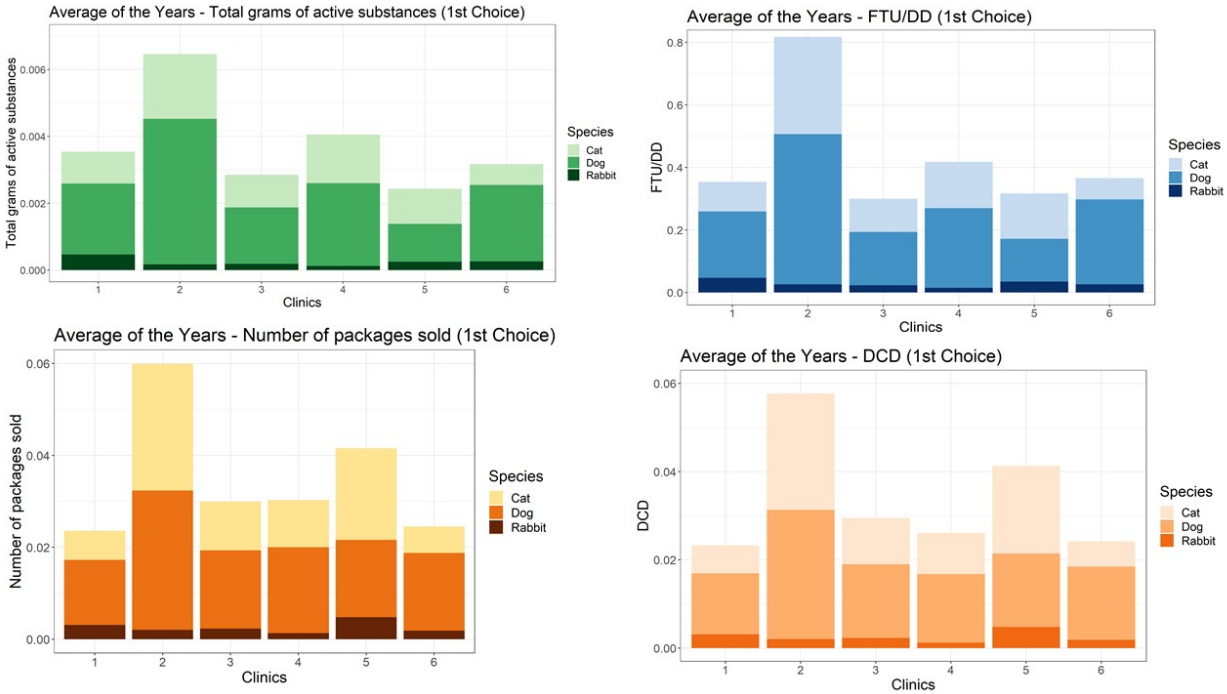


Figure 3: Outcomes of the four methods with regard to first choice topical antibiotics per species and per clinic on average in a year.

to that, in clinic number two the difference in exposure between cats and dogs is not so striking when quantifying using either one of the "Number of packages sold" or "DCD" method

whereas with the "Total grams of active substances" and "FTU/DD" methods, dogs come out as having been clearly exposed to more TAMs than cats.

The pattern of use concerning the choice of antibiotic with regard to the TAM products was similar for three out of the four quantification methods that were applied to the sample dataset. Particularly, with the quantification methods of "Total grams of active substances", "Number of packages sold" and "DCD", the topical antimicrobials that were used the most were second choice topical antibiotics, the second to have been used the most were first choice topical antibiotics and the least to have been used were third choice topical antibiotics. The "FTU/DD" method deviated from this pattern. Although second choice topical antibiotics came out as most used the relative use compared to first and third choice was much higher compared to the other quantification methods (Figure 4).

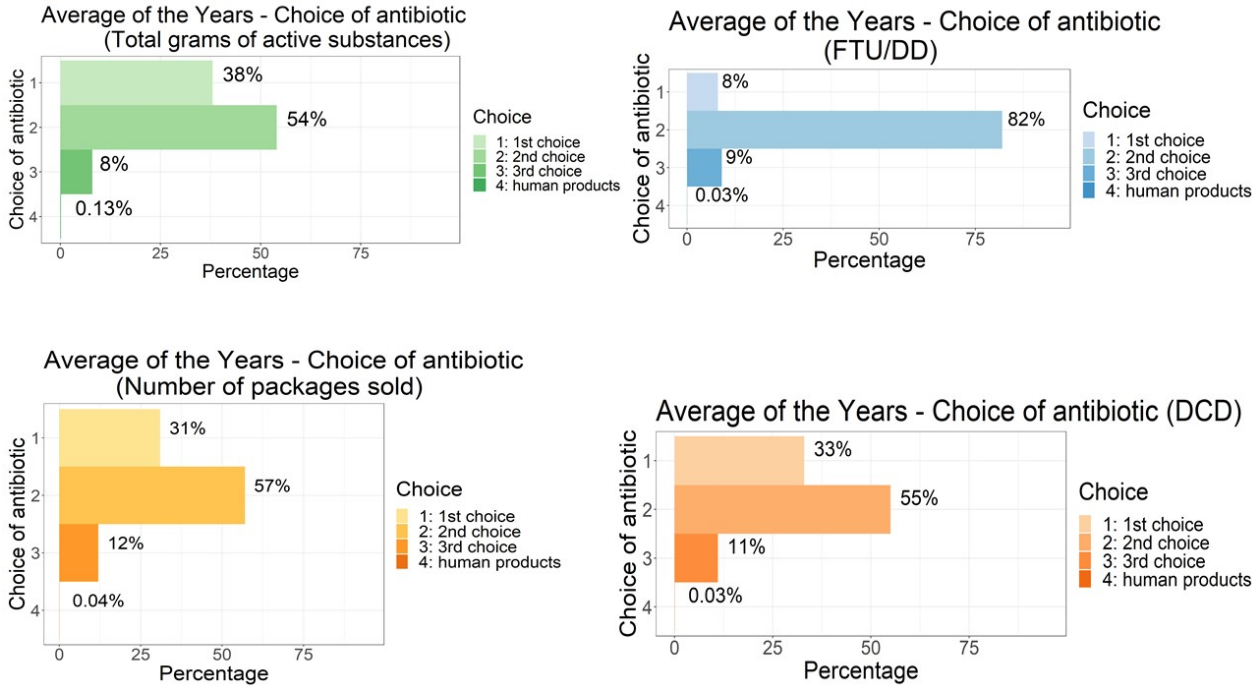


Figure 4: Outcomes of the four methods with regard to the choice of antibiotic on average in a year, expressed as a percentage.

The product types that came out as having been used the most on average in a year differed between most of the quantification methods with only the "Number of packages sold" and the "DCD" having similar outcomes. Specifically, after quantifying with these two methods, ear products came out as having been used the most on average in a year and second to have been used the most were eye products whereas skin products were used the least. Following the quantification with the "Total grams of active substances" method, eye products came out as having been used the most on average in a year, skin products were second to have been used the most while ear products were the least to have been used. Contrastingly, with the "FTU/DD" quantification method, ear products came out as having been used the most on average in a year, skin products were second to have been used the most and eye products were the least to have been used. Nasal products and products that were termed "unknown", which

included antimicrobials that are used in human medicine and intramammary products, were used in very low amounts in all the quantification methods. In Figure 5, a more detailed representation of the outcomes is presented.

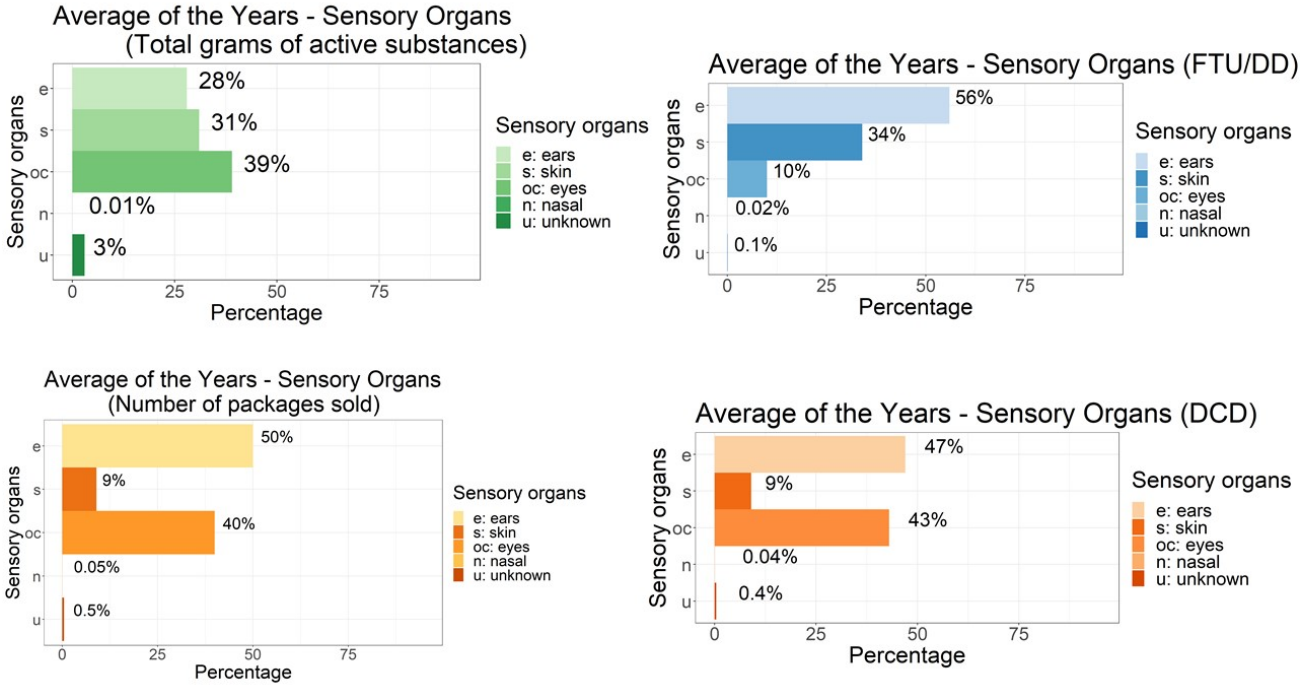


Figure 5: Outcomes of the four methods with regard to the sensory organ that the TAMs were used on, on average in a year, expressed as a percentage.

Stage 3: Experts' meeting

Four questions were asked to the experts in the survey that was implemented during the meeting. Each question asked the experts to order the quantification methods from the most suitable to the least, with regard to what each question inquired. Consequently, the placement of the methods would be from first place to fourth place. In Table 3, the questions that were in the survey, which the five experts answered during the meeting, are outlined as well as the ranking process for each respective question and their results.

Pertaining to the first question, which inquired which method was more appropriate for reflecting the actual use that occurs within a companion animal veterinary clinic, the "Number of packages sold" method was ranked as the most appropriate. In the second question, which inquired which method is the most easy to calculate, again the "Number of packages sold" method came out as the easiest to calculate. The last two questions, which inquired which method is the most appropriate to quantify the use of TAMs within and between clinics over time, the "Total grams of active substances" method was ranked as the most appropriate. Consequently, a consensus was not reached after the survey was completed since for the first two questions, the quantification method "Number of packages sold" was ranked highest and for the last two questions, the quantification method "Total grams of active substances" was the one that the experts ranked highest. An optional question, that the experts could answer

freely, was asked to the experts. This question asked whether the experts had other suggestions for another quantification method with regard to the quantification of topical AMU. Three experts answered this question, proposing that the DDDA method be used.

Following this, an open discussion was initiated with a primary focus on the DDDA method that was excluded in the first stage of this study. Particularly, the details on how the DDDA method could be used and adjusted for quantifying TAM use were discussed while the feasibility of applying this quantification method to the study's available data was also considered. Ultimately, the entirety of the five experts agreed that the DDDA method would be the most suitable method to quantify the use of TAMs in companion animal veterinary clinics.

Questions	Ranking	Results
1) Does the outcome of this quantification method **reflect the actual use** within the clinic?	Based on appropriateness (Most appropriate method should be placed on top)	1. Number of packages sold 2. Total grams of active substances 3. DCD 4. FTU/DD
2) Is this quantification method **easy** to calculate?	Based on ease of calculation (Most easy method should be placed on top)	1. Number of packages sold 2. DCD 3. Total grams of active substances 4. FTU/DD
3) Is this quantification method appropriate for comparing topical antimicrobial use **within** a clinic **over time**?	Based on appropriateness (Most appropriate method should be placed on top)	1. Total grams of active substances 2. DCD 3. Number of packages sold 4. FTU/DD
4) Is this quantification method appropriate for comparing topical antimicrobial use **between** clinics **over time**?	Based on appropriateness (Most appropriate method should be placed on top)	1. Total grams of active substances 2. Number of packages sold 3. DCD 4. FTU/DD

Table 3: Results of the survey implemented on the experts' meeting with regard to the four quantification methods.

Calculation process of the DDDA method

The DDDA method's calculation for TAMs is based on the average duration of the treatment and it is calculated as a fraction. Specifically, the numerator of this method is the number of packages of a specific TAM product that were dispensed by a clinic in a defined period of time multiplied by the Defined Daily Dose (DDD) assigned for this specific product. The DDD was equal to the assumed average duration of the treatment e.g. the number of days that the product should be applied to the companion animal for it to complete the treatment. This information was available in the SPC for some of the topical antimicrobial products whereas for the others, assumptions were necessary to be made by the QUANTA group. The rationale on the DDD assignment for the TAM products contained in the study's dataset and the specific DDDs used is included in Appendix IV. The DDDs were specified for each TAM product that was dispensed by the clinics in the dataset. Thus, the numerator is calculated as follows:

$$\text{Numerator} = \textit{Number of packages dispensed} \times \textit{DDD per product}$$

The denominator of this method is the animal population of the clinic as with previous methods. It is important to note that even though many products were not indicated for use in rabbits, based on the dataset of the six clinics, which contained the information of which TAM product was dispensed to which animal species, the great majority of TAMs were dispensed to rabbits regardless of not being indicated for use in this animal species. Additionally, the great majority of the products were indicated for use in both dogs and cats. Consequently, the denominator included the number of all three species that attended the respective clinics in the defined observation period.

$$\text{Denominator} = \text{Animal population} = \textit{Number of dogs} + \textit{number of cats} + \textit{number of rabbits}$$

As such, the full formula of the DDDA method for topical antimicrobials is as follows:

$$DDDA = \frac{\textit{Number of packages dispensed x DDD per product}}{\textit{Animal population}}$$

Advantages and disadvantages of the DDDA method

The DDDA method has the added advantage of being a more familiar method to veterinarians compared to the other four methods as it has been used in the livestock sector extensively (110) and recently, also in the companion animal sector (104) to quantify the use of systemic antimicrobials. In addition to that, this quantification method is easily calculated. Considering this study's dataset, the input data required to calculate the DDDA method were available and an additional advantage of this method is that it does not rely on the weight of the animals in order to be estimated nor on specific dosages. The disadvantages of this method consist of the assumptions that were necessary to be made with regard to the average duration of the treatment-and the DDD assignment-for certain TAM products for which this information was not available in the SPC. Another assumption that was made was that all TAM products are assumed to be dispensed to all animal species (dogs, cats and rabbits). Lastly, this quantification method does not account for "residual" products; TAMs that after the duration of the treatment has passed might be left in the respective bottles or tubes and might be used by the owners on other occasions. Nevertheless, specifically for certain types of TAM products such as eye drops and ear drops, it is advised not to be used after a long time has passed after the package has been opened.

Stage 4: Application of the final method

A strong seasonal effect was observed based on the results of the statistical model for the total topical AMU ($P < 0.001$). In particular, the statistical model suggests that the highest topical AMU occurred in the months July-August whereas the lowest in February-March. Regarding the seasonal effect, a similar pattern was observed for first and second choice topical AMU as well as for ear, eye and skin product AMU, which were also statistically significant ($P < 0.001$). In Figure 6, in the panels displaying the seasonal pattern of total, second choice and ear product

AMU, one clinic (grey line) appears to not follow the same seasonal pattern. However, this specific clinic rather than having three years of data had only two years and the topical AMU was consistently low in the month of June. This explains the exaggeration displayed in the figures, regarding this clinic. The lack of information coupled with the distinct low use in June creates the observed artifact in the graphs.

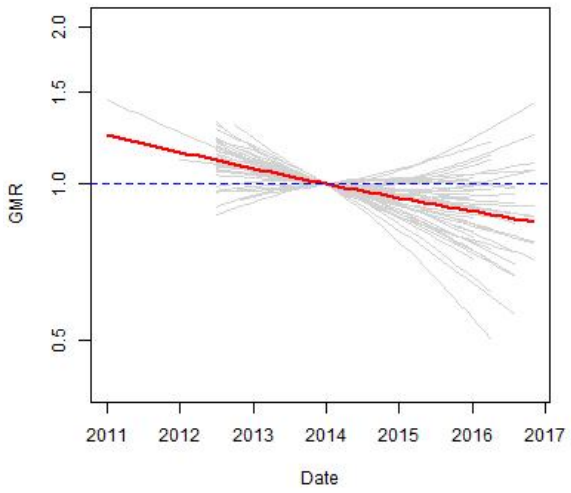
Furthermore, the statistical model indicated a significant shift in total topical AMU over time ($P < 0.001$), which suggested a decrease in total topical AMU from July 2012 until June 2015. Particularly, the mean total topical AMU in the first period (July 2012-June 2013) amounted to 0.927 DDDA/year whereas in the third period (July 2014-June 2015) the mean total topical AMU amounted to 0.835 DDDA/year. In Table 4, the mean total topical AMU for each year is displayed. The shift in first choice, ear and eye product AMU over time were also statistically significant ($P < 0.001$), indicating that a decrease occurred. Regarding second choice topical AMU, it appears that there is no substantial change in trend over time. In Figure 6, the trend over the years and the seasonal pattern is illustrated for the total, first and second choice TAMs and additionally for ear, eye and skin products.

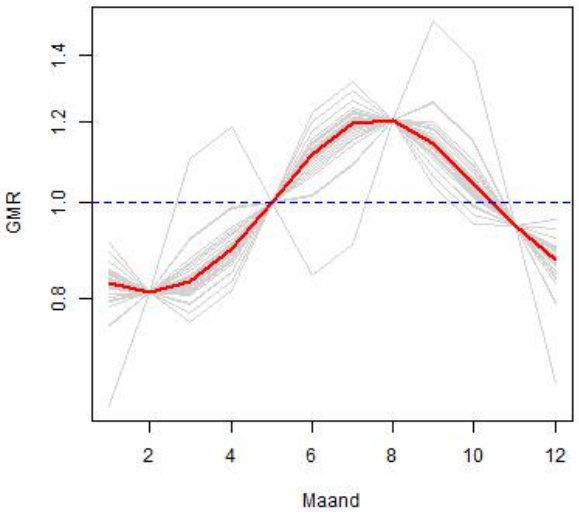
It is important to note that concerning third choice topical AMU as well as the AMU for nasal products and products for which their application is unknown in companion animals, the clinics did not provide many prescription data and that did not allow the estimation of the trend over time and the seasonality for these categories. Additionally, although skin products were prescribed by all 44 clinics, more data on ear and eye product prescriptions were available from the clinics.

	Period 1 (July 2012-June 2013)	**Period 2 (July 2013-June 2014)**	**Period 3 (July 2014-June 2015)**
Mean total topical AMU (DDDA/year)	0.927	0.904	0.835

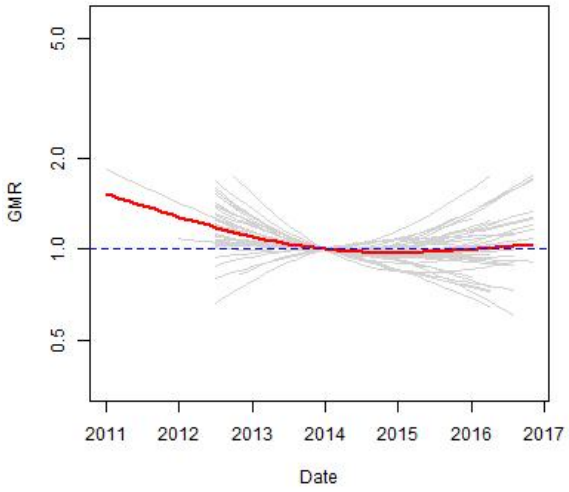
Table 4: Mean total topical AMU expressed in DDDA/year with regard to the 44 clinics that participated in the study. The mean is estimated for each period (e.g. each year; July 2012-June 2013, July 2013-June 2014, July 2014-June 2015) in the three-year study timeframe.

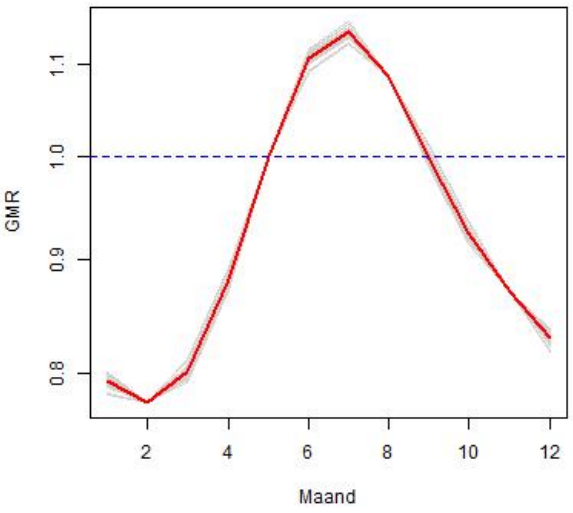
Total



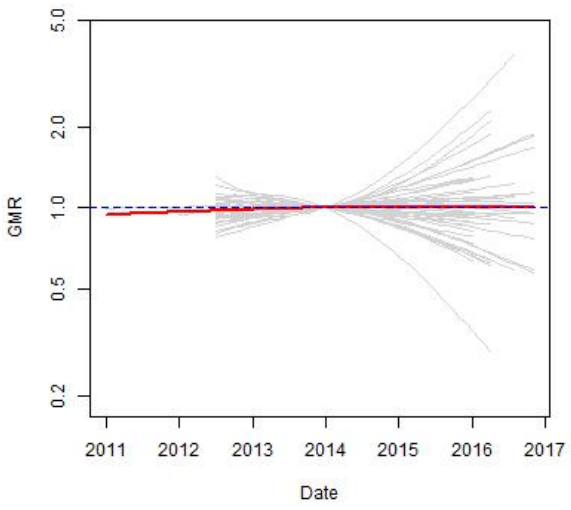


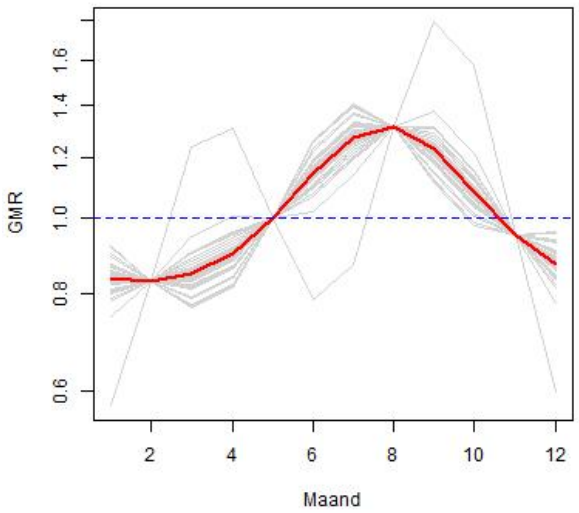
1st choice



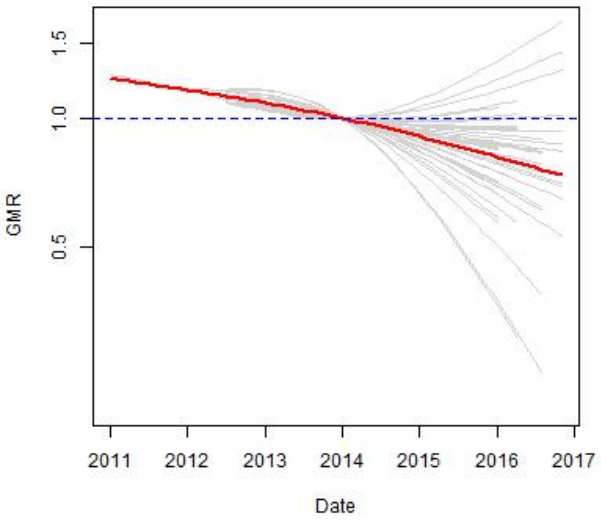


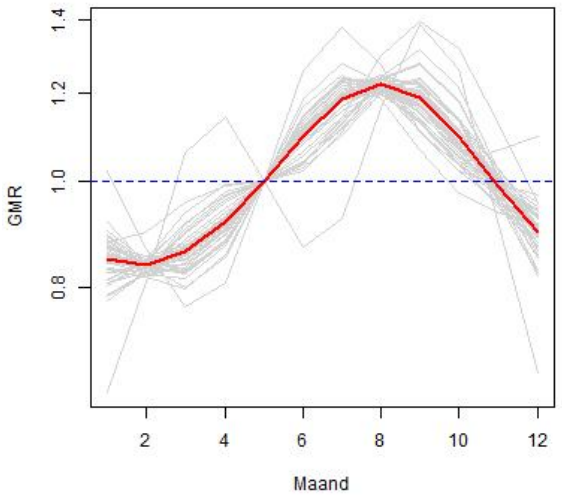
2nd choice





Ear products





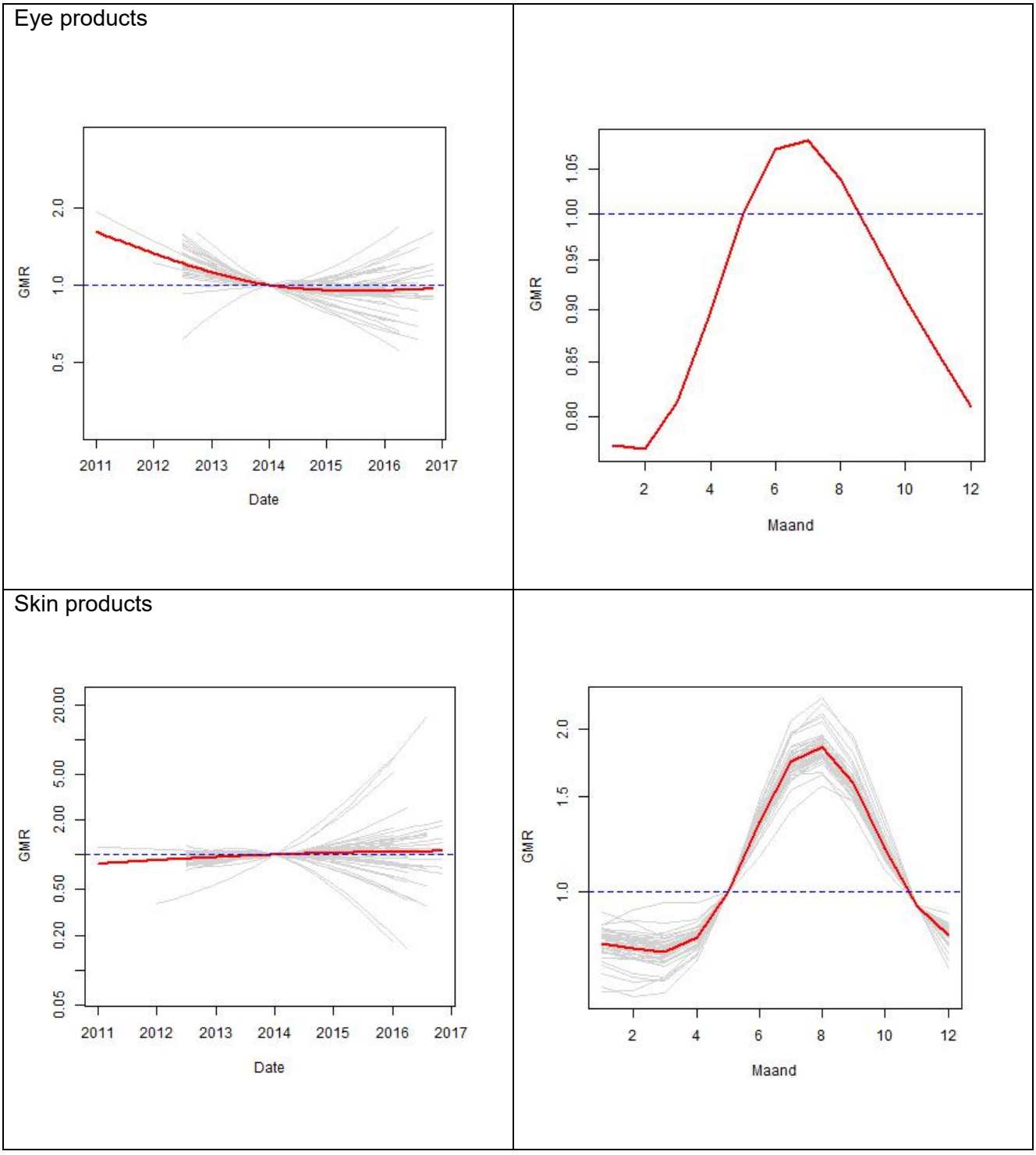


Figure 6: Time trend over the years during the period July 2012-June 2015 and seasonal pattern for different categories of topical AMU (*left and right panels, respectively)*. The categories are total, first, second choice topical AMU and ear, eye and skin product AMU. Regarding the time trend, the model produces a prediction in the respective topical AMU for the period after 2015. Both the trend in AMU over time and the seasonality are expressed as Geometric Mean Ratios (GMRs). The geometric mean in topical AMU in each year, concerning the trend over time, is compared to the average across the three-year period. The geometric mean in topical AMU in each month, regarding the seasonality, is compared to the average one across the year. Time trends were modelled using natural regression splines. The seasonal effect was modelled using harmonic functions. The mean of the random effects

distribution for both the time trend and the seasonal effect is displayed in red while in grey; the clinic specific estimates are shown. On the x-axis the year (Date) and the month (Maand) are indicated.

In the third period (July 2014-June 2015), determining the pattern of use regarding product types was possible for skin, ear and eye products. The reason for choosing this time frame was that 39 out of the 44 clinics could provide data on topical AMU for these product types in that period. As mentioned previously, there were not enough data for nasal or products for which their application was unknown in companion animals, thus these were excluded. Consequently, ear product AMU was found to be highest in that period with second highest being ear product AMU and third, skin product AMU. In Figure 7, this pattern is illustrated. This can only serve as an indication of the pattern of use concerning product types.

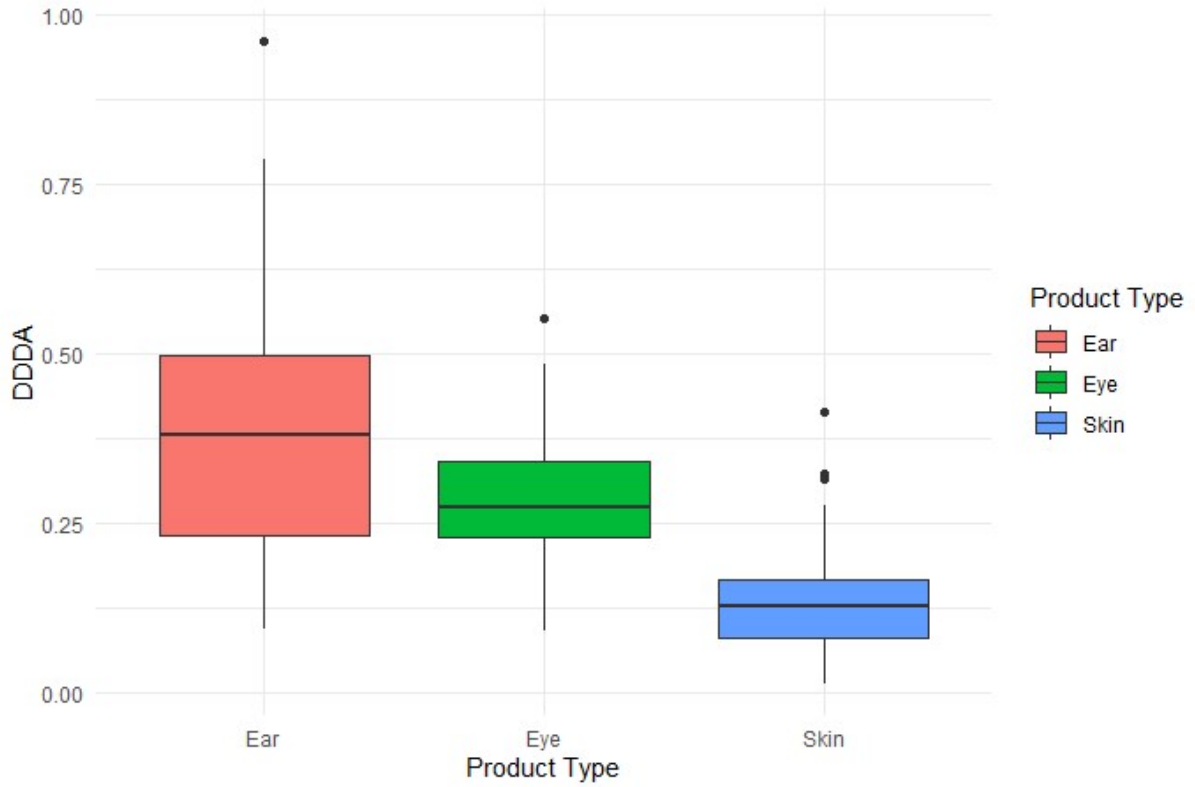


Figure 7: Boxplot displaying the average DDDA per year, concerning the use of ear, eye and skin products in period three (July 2014-June 2015) of the study. This is the outcome of data provided by 39 clinics.

Determinants of topical AMU

In Table 5, the estimates of the effect of potential determinants are presented.

A statistically significant positive association was discovered between total topical AMU and the proportion of dogs. This indicated that clinics with a larger proportion of dogs tended to have a higher total topical AMU (GMR 1.21, 95% CI 1.10-1.33 per 10% increase in the proportion of dogs). Particularly, this suggests that with each 10% increase in the proportion of dogs the ratio of geometric means increases by 21% for the total topical AMU. This was also significant for first and second choice topical AMU as well as for ear and skin product AMU (GMR for first choice topical AMU 1.14, 95% CI 1.01-1.28, GMR for second choice topical AMU 1.26, 95% CI 1.11-1.44, GMR for ear product AMU 1.32, 95% CI 1.14-1.52 and GMR for skin product AMU 1.53, 95% CI 1.17-2.01, per 10% increase in the proportion of dogs respectively). The rest of the determinants displayed no or almost significant associations with topical AMU.

CLINIC CHARACTERISTIC	TOTAL USE		FIRST CHOICE		SECOND CHOICE		EAR		EYE		SKIN	
	GMR	95%CI	GMR	95%CI	GMR	95%CI	GMR	95%CI	GMR	95%CI	GMR	95%CI
PROPORTION OF DOGS (PER 10% INCREASE)	1.21	**1.10-1.33***	1.14	**1.01-1.28***	1.26	**1.11-1.44***	1.32	**1.14-1.52***	1.09	**0.99-1.20****	1.53	**1.17-2.01***
PROPORTION OF RABBITS (PER 1% INCREASE)	0.97	0.93-1.02	0.97	0.91-1.03	0.96	0.90-1.03	0.99	0.92-1.06	0.99	0.94-1.04	0.91	0.79-1.04
TOTAL NUMBER OF ANIMALS (PER 1000)	0.97	0.90-1.04	0.97	0.89-1.06	0.97	0.88-1.07	0.96	0.86-1.07	1.00	0.93-1.08	1.08	0.88-1.32
NUMBER OF AFFILIATED PRACTICES	1.22	**0.98-1.51****	1.17	0.90-1.54	1.34	**0.99-1.81****	1.27	0.91-1.76	1.18	0.95-1.48	1.03	0.55-1.94
MEAN EXPERIENCE PER CLINIC (PER 10 YEARS)	1.02	0.87-1.20	1.02	0.83-1.25	0.92	0.73-1.16	0.92	0.72-1.18	1.04	0.88-1.24	1.29	0.80-2.08
URBAN (VERSUS RURAL OR MIXED)	0.82	0.55-1.22	0.96	0.59-1.57	1.09	**0.63-1.89***	0.83	0.46-1.51	0.75	0.50-1.13	1.58	**0.50-4.97***
CONVENTIONAL MEDICINE ONLY	1.23	0.95-1.59	1.29	0.94-1.78	1.60	1.11-2.29	1.37	0.93-2.02	1.07	0.82-1.40	2.27	**1.08-4.80**
GRADUATED IN UTRECHT	1.16	0.83-1.61	1.38	0.91-2.09	1.14	0.72-1.82	1.06	0.64-1.74	1.19	0.84-1.69	1.61	0.61-4.23
FEMALE VETERINARIANS ONLY	1.15	0.91-1.44	1.03	0.78-1.37	1.24	0.90-1.71	1.14	0.81-1.60	1.21	0.95-1.54	1.17	0.60-2.25
NOT SERVING SHELTERS/KENNELS	1.07	0.80-1.44	1.05	0.73-1.51	1.14	0.76-1.71	0.96	0.62-1.48	1.20	0.89-1.62	1.60	0.69-3.70
NOT SERVING BREEDERS	0.88	0.69-1.12	0.88	0.65-1.19	1.06	0.76-1.49	0.81	0.56-1.17	0.90	0.70-1.16	0.84	0.41-1.70
PMS 2 [1](VERSUS OTHERS)	0.98	**0.73-1.30***	0.81	**0.56-1.15***	1.05	0.70-1.58	1.00	0.65-1.55	0.91	**0.68-1.23****	1.34	0.58-3.09

[1]*PMS=Practice Management Systems type 2*, *P-value ≤ 0.05, **0.05< P-value ≤0.10

Table 5: Potential determinants of topical AMU. The results are the output of a multivariable regression model for total, first choice and second choice topical AMU as well as for ear and eye product AMU. The data are log-transformed.

Discussion

This study's principal objective was to identify a quantification method, which could be used to quantify the use of topical antimicrobials in a representative manner regarding the actual topical antimicrobial use that occurred in the respective companion animal veterinary clinics. To that end, four quantification methods were initially considered after a thorough search through relevant literature and a critical assessment of the discovered methods.

The four methods were the "Total Grams of active substances", the "Fingertip Unit/Droplet Dose (FTU/DD)", the "Number of packages sold" and the "Defined Course Dose (DCD or Number of treatment courses)". Nevertheless, none of these four quantification methods ended up as the best quantification method considering this study's objective, following their evaluation by a panel of experts. Contrastingly, the "Defined Daily Dose for Animals (DDDA)" adjusted for TAM products, which was excluded as a suitable quantification method at the first stage of the study, was deemed as the best to quantify the use of TAMs after the consensus agreement of the panel of experts at stage three of the study. This contradicts the WHO recommendation for the quantification of topical AMU, in which assigning DDDs is reported as not possible for dermatological preparations with the rationale that the amount given daily can vary substantially depending on the intensity and distribution of the condition (111). Thus, the WHO recommends expressing consumption in grams of preparations regardless of strength for these preparations (111). In this study, the FTU/DD quantification method falls in line with the WHO recommendation, however certain discrepancies were observed after quantifying topical preparations using this metric. Consequently, this method was ranked the lowest in the survey by the experts at stage three. Particularly, in the second stage of this study, when quantifying with the FTU/DD method, it was observed that second choice topical antibiotics were applied the most (i.e. 82% of the total topical AMU). A possible explanation for this is that second choice topical antibiotics were higher volume products, which based on the information included in the six-clinic dataset, was indeed the case. Thus, a quantification method, which is based on the volume of the preparations applied, such as the FTU/DD method, produces results that are analogous to the volume of the TAM products that were applied, which may lead to an over- or underestimation of the topical AMU. This method, specifically, has the additional disadvantage of being unknown to the veterinarians, as it has only been implemented in human medicine to quantify corticosteroid dermatological preparations (107). Consequently, familiarizing the veterinarians with calculating and, most importantly, interpreting a new quantification method might pose additional difficulties. This issue is avoided by choosing the DDDA method for quantifying the use of TAMs since this method and its interpretation is rather familiar to veterinarians as it is being used extensively in quantifying the systemic AMU in livestock and it has also been used in quantifying systemic AMU in companion animals (104,110,112).

Not many studies have tried to quantify topical AMU in companion animals. Certain studies measure the frequency of the systemic and topical AMU by using veterinary consultations extracted from electronic health records (EHR) (113,114). One study uses antimicrobial prescription data from veterinary practices to quantify systemic and topical AMU in the United

Kingdom (UK) (115). Joosten et al. use a simplified formula of the Treatment Incidence (TI) to quantify both systemic and topical AMU in Italy, Belgium and the Netherlands (116). According to the authors, the TI can be interpreted as "the percentage of a full year that the animal has been treated with a standard dose of antimicrobials" (116). This is the same as the DDDA method; however, the DDDA method used in this study to quantify topical AMU was estimated based only on the average days of treatment rather than the standard dose of the antimicrobials and did not make use of the weights of the animals. Instead, the total number of animals that attended the clinic on the observed time period was used. Contrastingly, Joosten et al. quantify topical AMU by taking into account the total duration of the treatment but also the recommended dose-based on the SPC information-and the standard weight of the animals (116). This way of estimating the TI (or DDDA) suggests that potentially more assumptions are necessary to be made. Finally, one study quantified topical AMU-in addition to quantifying systemic AMU-by using the number of treatments per 100 dogs and the number of treatments per 10 dog-years (97). Based on the study by Joosten et al. (116), they subsequently extrapolate their findings to DDDAs (97). Therefore, DDDAs have been used in quantifying topical AMU albeit using different calculation processes than what was used in the present study.

The Defined Daily Dose (DDD) quantification method constitutes a standardized quantification method that was developed by the WHO for reporting antimicrobial consumption for systemic antimicrobials in human medicine worldwide (102). This grants the opportunity of comparing data on AMU at the national and international level (102). Likewise, the European Surveillance of Veterinary Consumption (ESVAC) has published standardized DDDs for pigs, poultry and cattle (DDDvet) in nine European countries (99), which allows for an objective comparison of antimicrobial consumption between countries. Consequently, the DDDA method offers the possibility of an objective comparison of the AMU within and between clinics at a national or international level, allowing for trends and seasonal patterns to be explored over time (104). Quantifying the topical AMU with the same metric that systemic AMU is quantified introduces the possibility of comparing systemic and topical AMU. Although, the calculation process of the DDDA adjusted for TAMs is not the same as with the DDDA for systemic antimicrobials, essentially they measure the same entity; the exposure to the antimicrobials. Specifically, the DDDA method for TAMs is estimated based on the assumed average duration of the treatment with a specific TAM preparation. This designates the duration that a companion animal is exposed to a specific TAM. For systemic antimicrobials (AMs) in companion animals, Hopman et al. measure the exposure to these AMs by estimating the total treated animal weight and the total weight of the clinic animal population (104). However, the DDDA for TAMs does not require the weight of the animals in order to be estimated nor specific dosages of TAMs. Contrastingly, to quantify systemic AMU the average weight of the animals is used (104). Yet, incorporating the average weight of the animals in calculating the DDDA can be a cause of under- or overestimation of AMU (104), thus the fact that the DDDA for TAMs avoids using the weight of the animals constitutes an advantage of the method. Additionally, the specific dosages for TAMs are not necessary in estimating the DDDA for topical AMU, which is also advantageous since this information is lacking for most of TAM products. Still, certain assumptions were necessary to be made in

order to estimate the DDDA for TAMs. Firstly, an assumption was necessary in assigning DDDs for certain TAM products that were present in the dataset because the average duration of the treatment was not always included in the SPC. This could have potentially been mitigated by contacting again a panel of experts who could decide by consensus on the DDDs that should be assigned on specific TAM products that lacked this information in their SPC. However, due to time constraints the QUANTA group performed the DDD assignment. Additionally, it was assumed that all TAM products present in the dataset were prescribed to all three species that attended the clinics (dogs, cats and rabbits). The dataset contained patient-specific information only for six of the 44 clinics, thus solely these six clinics could indicate whether a specific TAM product was prescribed to a dog, a cat or a rabbit. The assumption to include all three species in the denominator of the DDDA for TAMs was made after inspecting the six-clinic dataset and observing that the great majority of the TAM products were indeed prescribed to all three species. Yet, despite these two assumptions, the DDDA method offers the unique possibility of comparing topical AMU data to systemic AMU data. This might permit the assessment of the topical AMU contribution to AMR, overall.

The DDDA method was used to describe and quantify the topical AMU that took place in the 44 Dutch companion animal veterinary clinics in the period from July 2012 until June 2015. A significant seasonal effect was discovered for total topical AMU and the same was true for first choice, second choice, ear, eye and skin product AMU. This indicated that the use peaked in the months of July-August and was lowest in the months of February-March. One clinic diverged from this seasonal pattern. However, this was an artefact. In particular, based on the information regarding the characteristics of this clinic, it appears to have had only one veterinarian employed, thus it is a possibility that this veterinarian went on vacation in June, potentially informing its clients to seek consultation in other clinics for that specific period of time. This might explain the low use observed in June in this clinic. Hopman et al. report a similar seasonal pattern but for systemic AMU, using the same dataset (94). In their study, they mention that a highest use might be observed in warmer months because certain conditions display a seasonality in their occurrence, such as allergic dermatitis which is observed more frequently in warmer months (94). In addition to that, Hopman et al. mention that in summer months, injuries from bite wounds or other dermatological issues might be more frequent (94). These could also explain the seasonal pattern that was found in this study, regarding topical AMU since TAMs are applied in dermatological conditions and in cases of otitis (84,117). Furthermore, in warmer months, owners and their pets might spend more time outdoors engaging in activities such as swimming in water bodies, which might predispose dogs for ear infections (118). In this study, additionally, it was indicated that ear products were used the most which could be explained by the aforementioned reasons, especially since otitis externa frequently manifests because of allergic dermatitis in dogs (119).

A significant change in total topical AMU from July 2012 until June 2015 was discovered in this study, suggesting that the use decreased. This was also statistically significant for first choice, ear and eye product AMU. Specifically, the mean total topical AMU decreased from 0.927 DDDA/year to 0.835 DDDA/year. Hopman et al. report a mean total AMU decrease,

concerning systemic antimicrobials, from 1.82 DDDA/year to 1.56 DDDA/year in the same period of time, using the same dataset (94). These estimations indicate that topical AMU might potentially contribute substantially to AMR emergence as the mean total topical AMU is approximately 50% of the mean total AMU regarding systemic antimicrobials. Méndez and Moreno report 5.3 and 8.1 DDDAs per year for topical and systemic AMU respectively, concerning companion animal veterinary practices in Madrid, Spain (97). This is the outcome of extrapolating their data to DDDAs (97). Singleton et al. as well as Mateus et al. report similarly substantial proportions of topical AMU, albeit using a different method to estimate AMU (114,115). Thus, it is indeed possible that topical AMU might play a considerable role in AMR emergence.

The proportion of dogs that were present in the clinics was significantly and positively associated with total topical AMU. This was also the case for first choice, second choice, ear, and skin product AMU. Thus, clinics with a higher proportion of dogs seemed to use more TAMs. Singleton et al. report that dogs were prescribed more topical antimicrobial agents compared to cats in a two-year study in the UK (114). Mateus et al. also report similar results (115). Singleton et al. argue that this result may possibly be explained by a better compliance of dogs regarding TAM applications and by the fact that dogs have a higher prevalence of certain dermatological diseases, such as pruritus compared to cats (114). These may also explain the observed results in this study.

Limitations

This study had certain limitations. Firstly, although the full dataset from the former ASAP project (94,95) was available, it was not possible to estimate the effect of the intervention to the topical AMU in the 44 Dutch companion animal veterinary clinics due to time constraints. Specifically, arriving at the final quantification method was a time-consuming process since initially four methods were considered and a fifth method (the DDDA) was introduced at a later stage in the study. Another limitation of the study was that only a few experts were available to participate in the survey (i.e. five experts). This made acquiring a consensus difficult through Mentimeter. Yet, the small number of experts facilitated the subsequent discussion, which resulted in choosing a quantification method for topical AMU. Furthermore, the proportion of first, second and third choice topical AMU could not be estimated because not all clinics had data on topical AMU in all the periods of the study. Particularly, the clinics reported very low amount of prescriptions with regard to third choice TAMs. The same was true for nasal products and products for which their application in companion animals was unknown.

Recommendations

It would be advantageous, in the future, to estimate the impact of the intervention on topical AMU as a continuation of the ASAP project (94,95). Thereafter, comparing the results between the topical AMU and the systemic AMU could shed more light in the overall effect of an intervention strategy on AMU and AMR as well as the dynamics between topical and systemic AMU within and between clinics. Additionally, the DDDA method could be further standardized by basing the DDD assignment on expert opinion.

Conclusion

Topical antimicrobial use can select for resistant strains of bacteria in both humans and companion animals. Consequently, a reduction in the efficacy of these antimicrobial preparations and the risk of negative therapeutic outcomes are troublesome. Although the transmission of antimicrobial resistant genes or resistant bacterial strains from humans to pets and vice versa remains complex to decipher, studies indicate the possibility of this occurring. Thus, quantifying the use of topical antimicrobials in companion animal veterinary clinics could provide insight on their contribution to the emergence and promotion of antimicrobial resistance as well as on broader characteristics concerning their use. This study presented the Defined Daily Dose for Animals (DDDA) method as suitable to quantify the use of topical antimicrobials in companion animal veterinary clinics. To current knowledge, this study was the first to determine the seasonality, time trends and determinants of topical AMU in 44 Dutch companion animal veterinary clinics over a three-year period using the DDDA method. In the future, better standardizing the DDDA method for topical AMU and applying it to estimate the intervention effect on topical AMU could offer more information on mitigation strategies regarding topical AMU and AMR.

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Supplementary Material

Appendix I

The QUANTA group consisted of:

- Nafsika Kardomatea, DVM
- Els Broens, DVM, PhD, Dipl. ECVM
- Nonke E. M. Hopman, DVM, PhD
- Ingeborg M. van Geijlswijk, PhD, PharmD

Appendix II

The DDDA method was also applied to the six-clinic dataset producing the following outcomes:

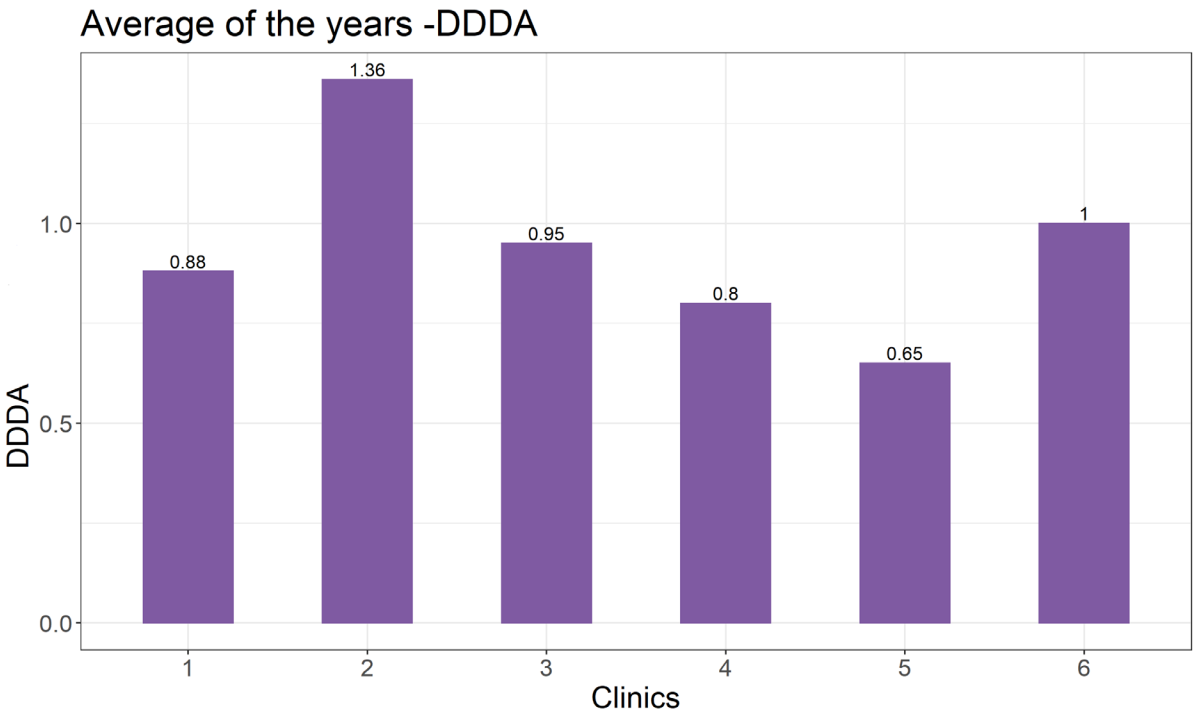


Figure 8: Outcome of the DDDA quantification method, indicating the average duration of treatment of an individual animal with a TAM on average in a year per clinic.

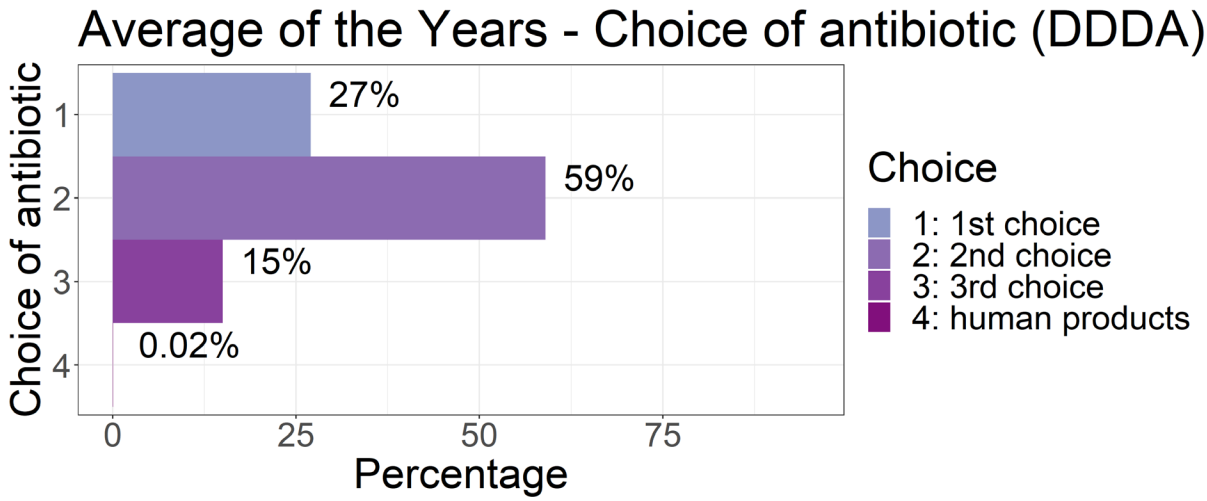


Figure 9: Outcome of the DDDA method with regard to the choice of antibiotic on average in a year, expressed as a percentage.

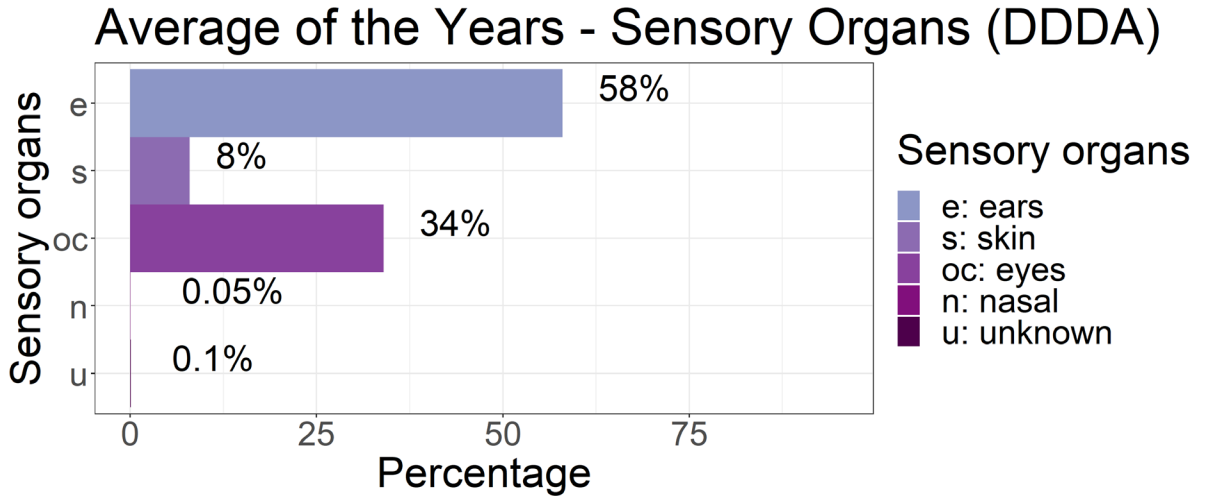


Figure 10: Outcome of the DDDA method with regard to the sensory organ that the TAMs were applied to, on average in a year, expressed as a percentage.

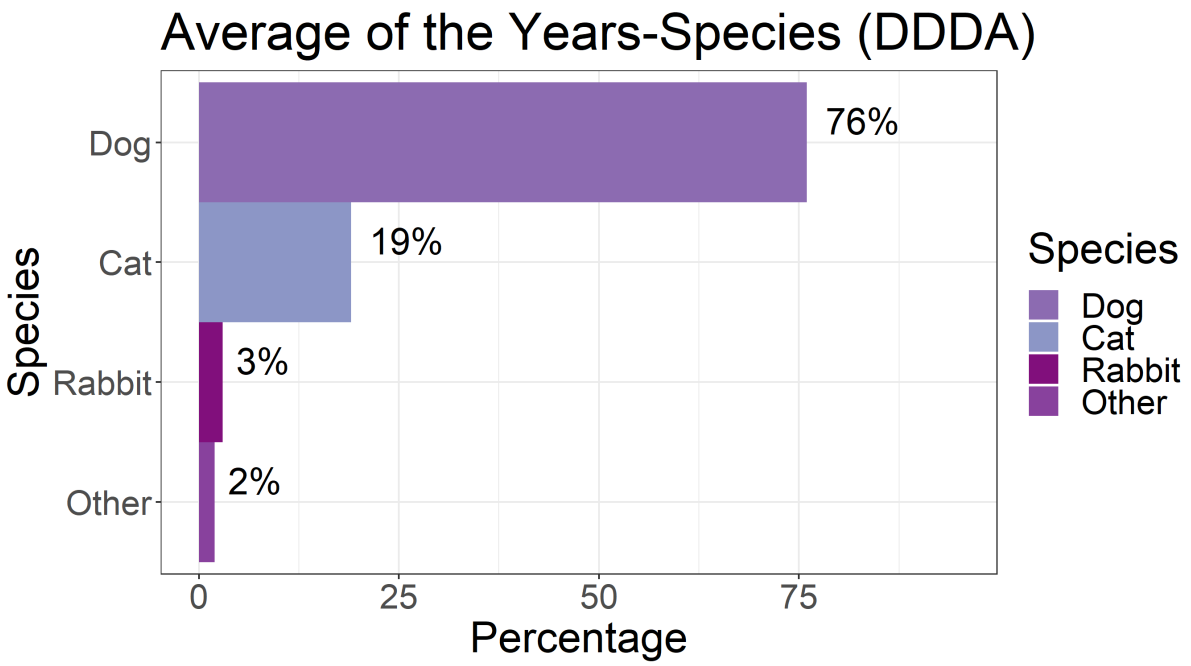
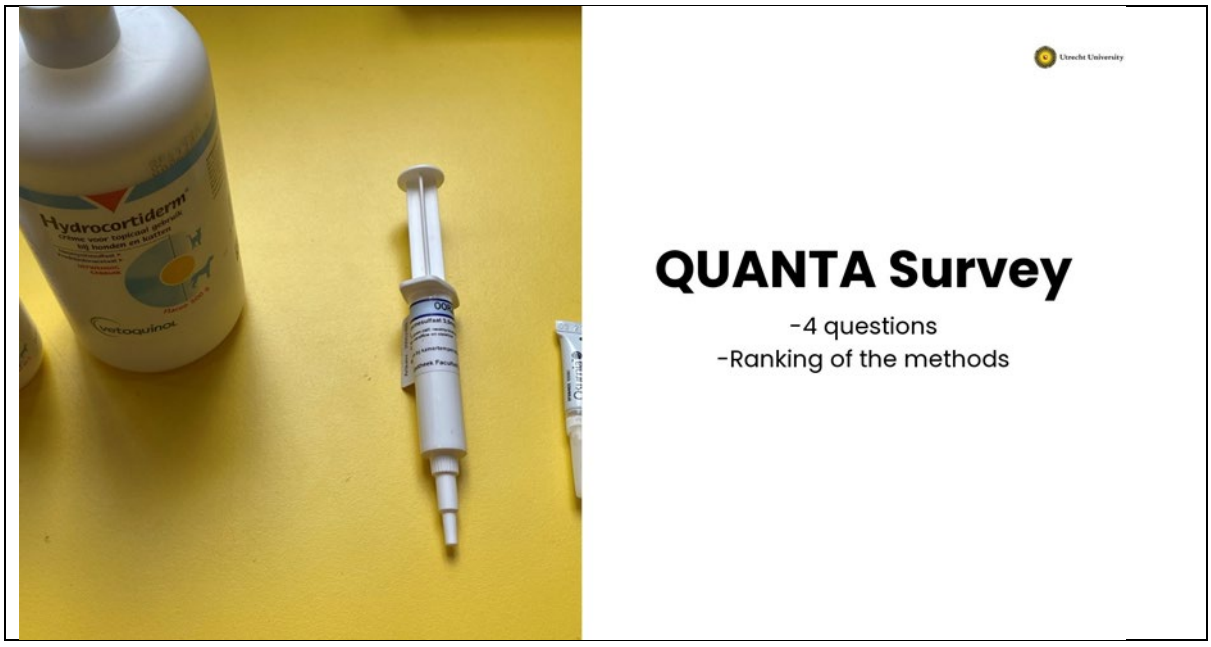


Figure 11: Outcome of the DDDA method with regard to the species that were treated with TAMs on average in a year, expressed as a percentage.

Appendix III

The survey as it was presented to the experts on the 23rd of June 2020 and the respective results are illustrated in this section. In Table 6, the details on how the experts ranked each method with regard to each question are presented.

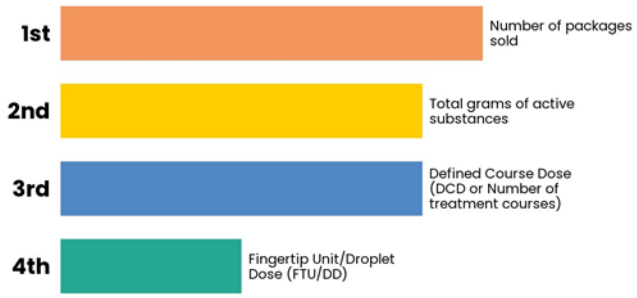


Q1- Ranking based on appropriateness:

Does the outcome of this quantification method **reflect the actual use** within the clinic?

(Most appropriate method should be placed on top)

This method best represents the actual topical AMU within a clinic



Q2- Ranking based on ease of calculation:

Is this quantification method **easy** to calculate?

(Most easy method should be placed on top)

Method 1: Total grams of active substances

$$Numerator = \frac{No\ of\ packages\ sold * package\ volume * strength}{1000}$$

mg → g

$$Denominator = animal\ population$$

*animal population= Number of dogs, cats and rabbits

$$Total\ grams\ of\ active\ substances = \frac{(No\ of\ packages\ sold * package\ volume * strength)/1000}{animal\ population}$$

Method 2: Fingertip Unit/Droplet Dose (FTU/DD)

$$Numerator = \frac{No\ of\ packages\ sold * package\ volume}{0.5}$$

*1 FTU/DD = 0.5 g (or ml)

$$Denominator = animal\ population$$

*animal population= Number of dogs, cats and rabbits

$$FTU/DD = \frac{\left[\frac{(No\ of\ packages\ * package\ volume)}{0.5}\right]}{animal\ population}$$

Method 3: Number of packages sold

$$Numerator = Number\ of\ packages\ sold$$

$$Denominator = animal\ population$$

*animal population= Number of dogs, cats and rabbits

$$Number\ of\ packages\ sold = \frac{No\ of\ packages\ sold}{animal\ population}$$

Method 4: Defined Course Dose (DCD or Number of treatment courses)

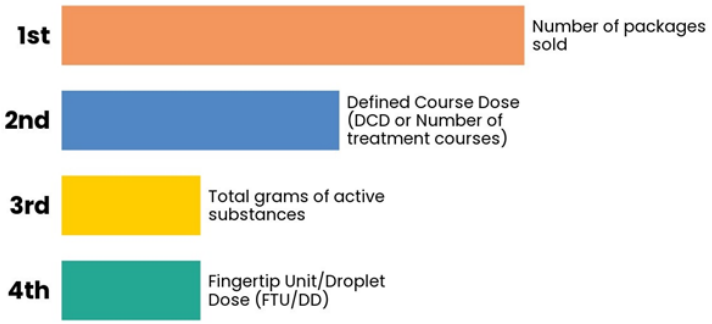
$$Numerator = Number\ of\ treatment\ courses$$

$$Denominator = animal\ population$$

*animal population= Number of dogs, cats and rabbits

$$DCD = \frac{No\ of\ treatment\ courses}{animal\ population}$$

This method is the easiest to calculate

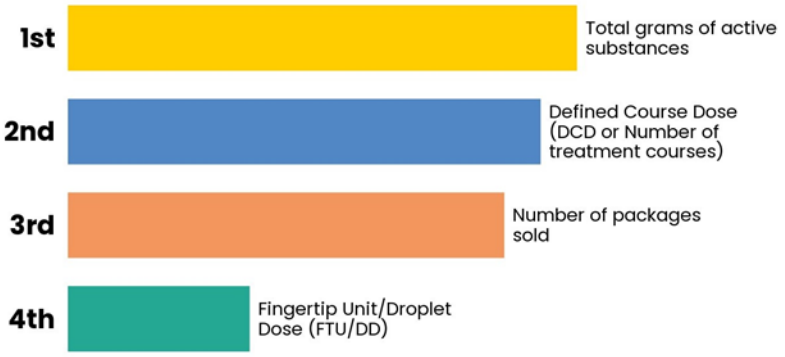


Q3-Ranking based on appropriateness:

Is this quantification method appropriate for comparing topical antimicrobial use **within** a clinic **over time**?

(Most appropriate method should be placed on top)

This method is the most appropriate to evaluate topical AMU within a clinic over time

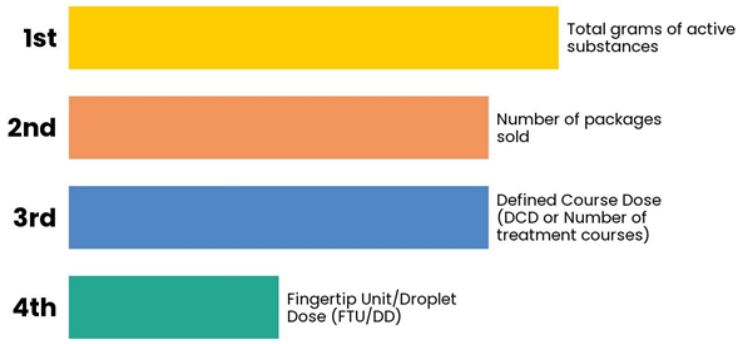


Q4-Ranking based on appropriateness:

Is this quantification method appropriate for comparing topical antimicrobial use **between** clinics **over time**?

(Most appropriate method should be placed on top)

This method is the most appropriate to compare topical AMU between clinics



Do you have suggestions for another quantification method?

Utrecht University

Defined daily dose

DDD

Animal day dosage specified for skin ear and eye

3

Question 1				
Date	2021-06-23			
Session	3			
Type	ranking			
Question	This method best represents the actual topical AMU within a clinic			
Respondents	5			
Items	**1st place**	**2nd place**	**3rd place**	**4th place**
Total grams of active substances	1	2	1	0
Fingertip Unit/Droplet Dose (FTU/DD)	0	0	2	2
Number of packages sold	2	2	0	0
Defined Course Dose (DCD or Number of treatment courses)	2	0	1	2

Question 2				
Date	2021-06-23			
Session	3			
Type	ranking			
Question	This method is the easiest to calculate			
Respondents	5			
Items	**1st place**	**2nd place**	**3rd place**	**4th place**
Total grams of active substances	0	0	2	2
Fingertip Unit/Droplet Dose (FTU/DD)	0	0	2	2
Number of packages sold	5	0	0	0
Defined Course Dose (DCD or Number of treatment courses)	0	4	0	0

Question 3				
Date	2021-06-23			
Session	3			
Type	ranking			
Question	This method is the most appropriate to evaluate topical AMU within a clinic over time			
Respondents	5			
Items	**1st place**	**2nd place**	**3rd place**	**4th place**
Total grams of active substances	3	0	1	0
Fingertip Unit/Droplet Dose (FTU/DD)	0	0	1	3
Number of packages sold	0	4	0	0
Defined Course Dose (DCD or Number of treatment courses)	2	0	2	1

Question 4				
Date	2021-06-23			
Session	3			
Type	ranking			
Question	This method is the most appropriate to compare topical AMU between clinics			
Respondents	5			
Items	**1st place**	**2nd place**	**3rd place**	**4th place**
Total grams of active substances	3	0	1	0
Fingertip Unit/Droplet Dose (FTU/DD)	0	0	2	2
Number of packages sold	0	4	0	0
Defined Course Dose (DCD or Number of treatment courses)	2	0	1	2

Table 6: The results of the experts' ranking process for each question in the survey.

Appendix IV

In Table 7, the DDDs that were assigned to the specific TAM products in the dataset that was used in this study are shown. The products that are highlighted red are the ones for which no information on the duration of the treatment was included in their SPCs. The QUANTA group considered 3 days of treatment (thus, DDDs) for spray and intramammary products. Regarding products that contained chloramphenicol, the QUANTA group considered 7 days of treatment. However, for one human product containing chloramphenicol the days of treatment were included in its SPC and for that one 10.5 DDDs were assigned accordingly. Yet, for the rest of the chloramphenicol products, the DDDs assigned were 7 because the QUANTA group deemed the 10.5 DDDs as a lot for these products. Concerning the product 'Opticlox', 4 days of treatment (thus, 4 DDDs) were considered because in the SPC it mentions that 1/15 of an injector is to be used four times per day. Regarding the product 'Fucithalmic', the QUANTA group considered 7 days of treatment (thus, 7 DDDs) since that was the case for other fucidic acid products applied in the eye. However, for 'Fucidine crème' the QUANTA group considered 6 days of treatment (6 DDDs) since this is the duration of the treatment of other fucidic acid products applied on the skin. Alternatively, for 'Fucidin intertulle', 3 days of treatment were considered (thus, 3 DDDs) because based on the information contained in the dataset that was used in this study, these products were sold per piece and not per package. The same DDDs as with the product 'Hydrocortiderm', thus 7 DDDs, were assigned to the products 'Sanoderm' and 'Dermapet' because they are all applied to the skin, are of similar volume and contain the same antibiotic substance. The QUANTA group considered that the product 'Maxitrol' contained a rather "intense" combination of antibiotic substances, thus 10 DDDs were assigned to this product. For the product 'Surolan', the same DDDs were assigned as with the product 'Aurimic' (thus, 10.5 DDDs) because they contain the same antibiotic substance and are used in the same manner. For the product 'Flammazine' and 'silver sulfadiazine', 10 DDDs were assigned because a healthcare professional leaflet that was discovered online mentioned that it should not be used for more than 14 days. Additionally, 7 DDDs were assigned to the product 'Terramycin' since it has the same antibiotic composition as 'Terra-cortil' for which the days of treatment were known from its SPC and they are both applied in the eyes. For the indication 'analklieren' the QUANTA group considered one day of treatment and so 1 DDD was assigned. Generally, if the information on the SPC for a specific TAM product mentioned a maximum treatment of 14 days, then the QUANTA group considered 10 days of treatment (or DDDs) for these products. The reasoning behind this is that taking the average is not sensible since there cannot be zero days of treatment whilst also 14 days of treatment is perhaps an exaggeration. If the SPC mentioned a minimum of 7 days of treatment then 7 DDDs were assigned.

Product	EAN-code	Comments	Dose (min) in mL	Dose (max) in mL	Times per day (min)	Times per day (max)	Duration (days-min)	Duration (days-max)	Species	Volume (g or ml)	DDDs (duration)	Antibiotic
Oogzalf Cavasan	8714646000578				4		until healed	until healed	Dog/Cat	5	7	Chloramphenicol
Oogzalf Cavasan	8714646000257				4		until healed	until healed	Dog/Cat	7.5	7	Chloramphenicol
Oordrupp. Surolan	8713241000433	ear & skin use		few drops		2	several days after disappearence of symptoms (2 to 3 weeks in some cases)	several days after disappearence of symptoms (2 to 3 weeks in some cases)	Dog/Cat	15	10.5	Polymyxine B sulfate
Oordrupp. Surolan	8713241001256	ear & skin use		few drops		2	several days after disappearence of symptoms (2 to 3 weeks in some cases)	several days after disappearence of symptoms (2 to 3 weeks in some cases)	Dog/Cat	30	10.5	Polymyxine B sulfate
Sanoderm	2016000000025										7	Neomycin sulfate
Ooggel Fucithalmic	5701170117691	drops	0.05	0.05	2	2	up to 2 days after disappearance of symptoms	up to 2 days after disappearance of symptoms	Human	3	7	Fucidic acid
Ooggel Fucithalmic	5701170117691	drops	0.05	0.05	2	2	up to 2 days after disappearance of symptoms	up to 2 days after disappearance of symptoms	Human	5	7	Fucidic acid
Oogzalf Terramycin tube	8715885000589				4	4			Dog/Cat	3.5	7	Oxytetracycline hydrochloride/Polymyxine B sulfate
Ooggel Isathal	5701170334821	drops	0.05	0.05	2	2	up to 2 days after disappearance of symptoms	up to 2 days after disappearance of symptoms	Dog/Cat	3	7	Fucidic acid
Caf oogzalf	8715442001134				4	4	until healed	until healed	Dog/Cat	5	7	Chloramphenicol
Caf oogzalf	8715442001141				4	4	until healed	until healed	Dog/Cat	10	7	Chloramphenicol
Chloramfenicol oogzalf + vit A	8714076000599				4	4			Dog/Cat	5	7	Chloramphenicol
Curaclox inj	8714225013357										2	Ampicillin sodium/Cloxacillin sodium
Maxitrol oogdruppels	2016000000004	drops	0.05	0.1	4	6			Human	5	10	Polymyxine B sulfate/Neomycin sulfate
Maxitrol oogdruppels via RUU	2016000000002	drops	0.05	0.1	4	6			Human	10	10	Polymyxine B sulfate/Neomycin sulfate
A.A.-Opthacaf oogdruppels	8714646000578				4	4			Dog/Cat	5	7	Chloramphenicol
A.A.-Opthacaf oogdruppels	2016000000036				4	4			Dog/Cat	10	7	Chloramphenicol
Avuloxil injectoren (Amx+clavul)	8715885000022										2	Amoxicillin trihydrate/Clavulanic acid
Anaalklieren spoelen incl zalf											1	
Chloramph.oogzalf+vit.A	8714076000599	ointment			4				Dog/Cat	5	7	Chloramphenicol
Prevaclox injector	8714225013265										2	Ampicillin/Cloxacillin Bezathine
Super mastidol	8714076004757										2	Benzylpenicillin potassium/Benzylpenicillin procaine/Neomycin Sulfate
Ubrolexin 10 injectoren	8711642013519										2	Cefalexin monohydrate/Kanamycin monosulphate
Chlooramfenicol CAF Oogzalf + Vitamine	8715442001141										7	Chloramphenicol
CTC spray	8714225151967								Cattle/Sheep/Pig	422	3	Chlorotetracycline hydrochloride
CTC spray	8714225013159								Cattle/Sheep/Pig	211	3	Chlorotetracycline hydrochloride
oorzalf AST	2016000000002										2	Neomycine sulfate
oorzalf	2016000000002										2	Neomycine sulfate
Alamycin aerosol	5023534100065								Cattle/Sheep/Pig	140	3	Oxytetracyline hydrochloride
Flammazine creme 1%	2016000000033			2-3 mm thick application	1	1			Human	50	10	Silver sulfadiazine
Zilversulfadiazine	2016000000033			2-3 mm thick application	1	1			Human	50	10	Silver sulfadiazine
Fucidin creme	5701170334265				3	4			Human	15	6	Fucidic acid
Animedazon spray	8714646001681								Cattle/Sheep/Pig	211	3	Chlorotetracycline hydrochloride
Engemycine spray	8713184093233								Cattle/Sheep/Pig	200	3	Oxytetracyline hydrochloride
Opticlox eye ointment	5023534101567			1/15 injector of 5 g per eye	4	4	until healed	until healed	Dog/Cat/Horse/Cattle/Sheep	5	4	Cloxacillin Bezathine
Dermapet	8714225004980										7	Neomycin sulfate

Cobactan lc	5412734000525								Lactating cows		2	Cefquinome sulphate
Oordruppels Auribiotic	8714646000486	Ear use, drops	0.15	0.3	2	3	5	10	Dog/Cat	20	7.5	Neomycin Sulfate
Oordruppels Auribiotic	8714646000486	Eye use, drops	0.05	0.1	4	6	5	10	Dog/Cat	20	7.5	Neomycin Sulfate
Oordruppels Auribiotic	8714646000882	Ear use, drops	0.15	0.3	2	3	5	10	Dog/Cat	50	7.5	Neomycin Sulfate
Oordruppels Auribiotic	8714646000882	Eye use, drops	0.05	0.1	4	6	5	10	Dog/Cat	50	7.5	Neomycin Sulfate
Oogzalf Terra-cortril	8713332001684	drops	0.10	0.15	2	3		14	Human	5	7	Oxytetracycline hydrochloride/Polymyxine B sulfate
Oogdruppels Gentapol-b	8714646000455	drops	0.05	0.1	4	4	3	5	Dog/Cat	5	4	Gentamycin sulfate/Polymyxine B sulfate
Rhinigenta drops	8714646000523	drops	0.10	0.2	6	6	5	10	Dog/Cat	10	7.5	Gentamycin sulfate
Oordruppels Aurizon	8715287001320	drops	0.50	0.5	1	1	7	14	Dog	10	10.5	Marboflocaxin
Oordruppels Aurizon	3605877140469	drops	0.50	0.5	1	1	7	14	Dog	20	10.5	Marboflocaxin
Aurizon	8715287001917	drops	0.50	0.5	1	1	7	14	Dog	20	10.5	Marboflocaxin
Oordruppels Easotic	8714076004733	ml (dose)	1.00	1	1	1	5	5	Dog	10	5	Gentamycin sulfate
Clindacutin zalf	8714646000110				3	4	7	14	Dog	60	10.5	Clindamycin hydrochloride
Clindacutin zalf	8714646000660				3	4	7	14	Dog	20	10.5	Clindamycin hydrochloride
Clindacutin zalf	8714646002435				3	4	7	14	Dog	50	10.5	Clindamycin hydrochloride
Clindacutin injector	8714646000103				3	4	7	14	Dog	10	10.5	Clindamycin hydrochloride
Otiderm oordruppels	8714646001377	drops	0.50	0.5	1	1	7	14	Dog/Cat	10	10.5	Enrofloxacin
Otiderm oordruppels	8714646001438	drops	0.50	0.5	1	1	7	14	Dog/Cat	20	10.5	Enrofloxacin
Hydrocortiderm	3605870003228				2	3		14	Dog/Cat	60	7	Neomycin Sulfate
Hydrocortiderm	3605870003259				2	3		14	Dog/Cat	120	7	Neomycin Sulfate
Neonystatriam oorzalf	2016000000003										2	Neomycin Sulfate
Oculsan	8714646000493	drops	0.05	0.1	4	6	5	10	Dog/Cat	5	7.5	Neomycin Sulfate
Gentapolycort oogdruppels	8714646000530	drops	0.10	0.1	3	4	3	5	Dog/Cat	5	4	Gentamycin sulfate/Polymyxine B sulfate
Neoticort oorzalf	2016000000019										2	Neomycin Sulfate
Neom./nyst/triam.oorzalf	2016000000003			fill ear canal	1	1			Dog/Cat/Horse	5 tubes of 10 g	2	Neomycin Sulfate
Tiacil, oogdruppels	8714076002340	drops	0.05	0.1	3	3	5	7	Dog/Cat/Rabbit	5	6	Gentamycin sulfate
Neomycine/triam. oorzalf	2016000000002			fill ear canal	1	1			Dog/Cat/Horse	5 tubes of 10 g	2	Neomycin Sulfate/triamcinolonac
Aureomycin oogzalf	8714646000653				4	6	3	5	Dog/Cat/Horse	5	4	Chlorotetracycline hydrochloride
Otifin PD oordr	8715442001455	drops	0.15	0.3	2	2	7	14	Dog/Cat	20	10.5	Gentamycin sulfate/Polymyxine B sulfate
Chloramphenicol oogdr 0,5%	8715442001141	drops	0.05	0.1	8	12	7	14	Human	10	10.5	Chloramphenicol
Trafloxal oogdr	2016000000008	drops	0.05	0.05	4	4		14	Human	5	7	Ofloxacin
Helgritin P.D oogdr	8715442001332	Eye use, drops	0.05	0.1	3	4	3	5	Dog/Cat	5	4	Gentamycin sulfate/Polymyxine B sulfate
Helgritin P.D oogdr	8715442001332	Ear use, drops	0.15	0.3	2	2	7	14	Dog/Cat	5	10.5	Gentamycin sulfate/Polymyxine B sulfate
Neoticort	2016000000019										2	Neomycin Sulfate
Isaderm	5701170334265			0.5 cm length of gel per 8 cm2 of lesion	2	2	5	7	Dog	15	6	Fucidic acid
Neoticort plus oorzalf	2016000000020										2	Neomycin Sulfate
Neoticort+nystatine	2016000000020										2	Neomycin Sulfate
Osurnia	2016000000016			1 tube per infected ear		repeat after 7 days	2 administrations (7 days between them)	2 administrations (7 days between them)	Dog	2/ 12/20/40 tubes (each tube is 1.2 g)	7	Florfenicol
Vetaderm	87146460100003	2 spray depressions of 0,7 ml each	1.40	1.4	2	4	7	7	Dog	60	7	Neomycin sulfate
Vetaderm	87146460101570	2 spray depressions of 0,7 ml each	1.40	1.4	2	4	7	7	Dog	120	7	Neomycin sulfate

Vetaderm	87146460101640	2 spray depressions of 0,7 ml each	1.40	1.4	2	4	7	7	Dog	500	7	Neomycin sulfate
Clindobion wond/huidzal	8715442001172				3	4	7	14	Dog	20	10.5	Clindamycin hydrochloride
Dermalgin	87154420010111	2 to 3 daily/ 2 to 3 weekly			2	3		14	Dog/Cat	60	7	Neomycin Sulfate
Dermalgin	87184442701641	2 to 3 daily/ 2 to 3 weekly			2	3		14	Dog/Cat	120	7	Neomycin Sulfate
Polymyxine B Oorzalf	2016000000011			fill ear canal	1	1	7	7	Dog/Cat	7	2	Polymyxine B sulfate
Polymyxine B Oorzalf	2016000000040			fill ear canal	1	1	7	7	Dog/Cat	7	2	Polymyxine B sulfate
Neotriam oorzalf	2016000000002			fill ear canal	1	1	7	7	Dog/Cat	7	2	Neomycin Sulfate
Posatex oordr.	8713184100139	drops	0.10	0.4	1	1	7	7	Dog	8.8	7	Orbifloxacin
Posatex oordr.	8713184102249	drops	0.10	0.4	1	1	7	7	Dog	35.1	7	Orbifloxacin
Polytriam oorzalf	2016000000011				1	1	7	7	Dog/Cat	7	2	Polymyxin B sulfate
Aurimic oordruppels	2016000000007	drops	0.25	0.25	2	2	7	14	Dog/Cat	20	10.5	Polymyxin B sulfate
Neomycine oorzalf	2016000000002				1	1	7	7	Dog/Cat	7	2	Neomycin Sulfate
Ciprofloxacine 3mg/g oorgel	20160000000104	drops, every 2 hours (first 2 days) then every 4 hours	0.05	0.05	6	12		7	Human	5	3.5	Ciprofloxacin
Bactroban zalf	2016000000017				2	3		10	Human	15	5	Mupirocin
Sofradex oogdruppels	2016000000026	drops,1 to 2 hours but after 2 to 3 days reduce to 3 to 4 times a day	0.05	0.1	3	4		7	Human	8	3.5	Framycetin sulfate/Gramicidin
Canaural oordruppels	2016000000031	drops	0.25	0.5	2	2	7	7	Dog/Cat	15	7	Fucidic acid/Neomycin B
Canaural oordruppels	2016000000032	drops	0.25	0.5	2	2	7	7	Dog/Cat	25	7	Fucidic acid/Neomycin B
Fuciderm	5701170334265				2	2	5	7	Dog	15	6	Fucidic acid
Fucidin intertulle	2016000000049			1 gauze	1	1 (later after 2 to 3 days)	7	14	Human	box of 10 and 50 pieces (10x10 cm)	3	Fucidic acid
Tobradex oogdruppels	2016000000030	drops,every 2 hours (first 1-2 days) then reduced (severe cases)/ 4 to 6 times (less severe)	0.05	0.1	4	6		14	Human	5	7	Tobramycin

Table 7: Table displaying each TAM product in the dataset, the information retrieved from its SPC and the DDDs that were assigned to them.