Inventory of the use of antiparasitic drugs in dairy cattle in the Netherlands

Master Thesis Veterinary Medicine
Utrecht University

Frouke R. Attema
6435653
30-01-2024

Supervisors
Dr. R. Jorritsma
Dr. I.M. van Geijlswijk
Dr. T. van Werven

Table of contents

1. Abstract

Antiparasitic drugs exist to reduce the negative effects of parasitic infections. The use of these drugs has negative side effects, such as the risk of antiparasitic drug resistance and negative effects on the environment. To estimate the extent of these effects, it is necessary to know how often and which antiparasitic drugs are administered. This is the first study to quantify the use of antiparasitic drugs in dairy cattle in the Netherlands. This is done by translating the sales data of the Universitaire Landbouwhuisdieren Praktijk (ULP) into defined daily dosages per animal (DDDA). A total of 273 farms is included in this study. To evaluate the antiparasitic drug use on farm level, the $DDDA_F$ was calculated. For drugs authorized for specific age categories, the $DDDA_S$ was calculated, using the number and weight of the corresponding age categories. Two different $DDDA_S$ were calculated, the $DDDA_S$ 'Cattle aged <2' and the $DDDA_S$ 'Cattle aged <1'. The seasonal distribution was made visible by showing the DDDA values per quarter. An indication of a seasonal effect in the use of antiparasitic drugs was found, however, to confirm this effect, it should be studied more extensively. The DDDA values of the population show a wide spread, on farm level as well as in the specific categories. A large part of the population does not even use antiparasitic drugs at all. The wide spread together with the number of no use farms indicates that an overall reduction is possible a reduction with the goal of preserving the ability to treat infected animals and minimize the effects for the environment.

2. Introduction

Cattle can be infected with various kinds of parasitic infections. Ranging from endoparasites in the gastrointestinal tract such as the nematode *Ostertagia ostertagi* or endoparasites in the respiratory system such as the nematode *Dictyocaulus viviparus* to ectoparasites such as the louse *Bovicula bovis*. Parasite infections can cause clinical signs such as anorexia, weight loss, diarrhoea, dyspnoea[1], and itching[2]. In addition to this, insects can act as vectors for arboviruses, such as the Bluetongue virus[3]. Overall, these clinical signs result in loss of animal welfare and an have economic impact due to decreased growth, milk yield, and fertility[4].
To eliminate these infections for the purpose of reducing the negative effects of these infections, several veterinary medicinal products (VMP's) of different pharmacotherapeutic groups are available. While several non-medicinal measures can be taken as well, such as for instance repeated moves to clean pasture[5] or vaccination[6], currently, the livestock industry is still relying heavily on these drugs[7]. This reliance on antiparasitic drugs has led to an extensive usage. This, combined with improper dosage and other factors has resulted in drug resistance. Resistance in parasites to anthelmintics is defined as the genetically transmitted loss of sensitivity in worm populations that were previously sensitive to the same drug. When antiparasitic drugs are administered, they selectively remove the susceptible worm individuals from the genetically heterogeneous populations. This leads to an increase of worm individuals that carry genes conferring drug resistance. These genes are passed on to their offspring and this way resistance genes can accumulate over several generations[8]. Or when improper dosages are applied, parasites are exposed to sub-lethal dosages which increases the risk of selection[9]. Altogether, parasitic drug resistance causes serious threats to the effective control of infections[8].
But drug resistance is not the only negative consequence of the administration of antiparasitic drugs. After administration, all antiparasitic drugs eventually have to be excreted. This way, residues of these drugs end up in the environment. Even though every different group of antiparasitic drugs has a different mechanism of action, they all have the effect of either killing or paralysing parasites[8;9]. These effects are not specific for the pathogenic parasites in and around the animal, but apply to other species in the environment as well[10]. When a large part of the dose is unchanged excreted in the faeces, it means that the excreted drug can reduce the number of insects in the dung of the cow[11]. This so-called toxic non-target effect negatively affects the ecosystem[12], for example because a large number of insects is essential for dung degradation[13]. These insects are part of the dung organisms. These organisms spend a part or all of their life in close association with dung pats. Dung organisms therefore include for example earthworms and dung feeding species such as coprophagous beetles and flies[14]. When the number of dung organisms is reduced, a reduction may occur in the feeding and tunnelling activities of dung-dwelling insects, which may delay dung degradation. This in turn may result to an increase in pest flies, because these undegraded dung pats provide sites for these pest flies to complete development[13].
A different form of life that is affected by antiparasitic drugs are pollinators. Pollinators, for example bees, serve as bio-monitor tools. They are essential for the reproduction of many plants and for sustaining ecosystems. When livestock are treated with veterinary medicinal products, these pollinators can come in contact with the excreted active metabolites of these products, for example via water or soil. While there is a need for more research on the exact effect of antiparasitic drugs on bees, it is clear that antiparasitic drugs such as avermectins and pyrethroids have negative effects on pollinators[15]. These effects range from neurotoxicity[16] to impairing the olfactory memory[17] and can even result in mortality.
As different groups of antiparasitic drugs have different mechanisms of actions, their side effects can be different as well. A closer look will be taken on anthelmintics, pyrethroids and antiprotozoal drugs.

2.1 Anthelmintic drugs

Anthelmintic drugs are administered to control infections with helminths such as gastrointestinal nematodes and liver flukes. Various pharmacotherapeutic groups fall under the classification of anthelmintic drugs, the most important being benzimidazoles, imidazothiazoles and macrocyclic lactones[18]. This last group is classified as endectocides, meaning that, in addition to being effective against endoparasites, these substances are effective against ectoparasites as well[13].

In small ruminants, and especially in sheep, resistance to antiparasitic drugs, anthelmintics in particular, is a well-known problem[19]. It has been established that in the Netherlands anthelmintic resistance amongst small ruminants is widespread and involves products from almost all major anthelmintic classes. In a study[20] in the Netherlands, it was found that ivermectin resistance occurs most frequently. In this study, resistance to this drug was found in 78.3% of the participating flocks. In addition to this, high levels of resistance were found to oxfendazole and moxidectin. In 47.1% of the flocks, parasites expressing resistance to multiple drugs were found. This illustrates the extent of the problem in the small ruminant industry.

Although the extent of the problem of anthelmintic resistance in the cattle industry is less evident, this does not mean that it is of less importance. Anthelmintic resistance amongst cattle is a global problem. A meta-analysis in 2020[18] established that anthelmintic resistance is present across the European continent in all ruminant species. The prevalence varied widely between the classes, with resistance to benzimidazoles and macrocyclic lactones ranging from 0 to 100%, resistance to levamisole ranging from 0 to 17% and resistance to moxidectin ranging from 0 to 73%.

In the Netherlands anthelmintic resistance was reported for the first time in the previous century. In the winter of 1998/1999 a farm located in the Dutch province of Noord Holland was found to have triclabendazole-resistant *Fasciola hepatica* infected cattle[21]. A decade later, RoyalGD conducted a study[22] to assess the situation in the Netherlands at that time. Even though no resistance was found amongst lungworms and most of the pathogenic gastro-intestinal nematodes, there was evidence of decreased effectiveness in the treatment of the less pathogenic gastrointestinal worms and liver fluke. The term decreased effectiveness is granted when treatment results in only a partial reduction of egg excretion. This indicated that resistance to anthelmintics was present in the Netherlands, and it was speculated that this problem will only worsen in the future when no restrictive measures are taken.

The metabolism of anthelmintic drugs varies per drug. In general, the characteristics of the drug combined with the time and frequency of application strongly influence the route of excretion in the environment[23]. To illustrate the metabolism of anthelmintic drugs, ivermectin serves as an example, as this is a widely used drug. Ivermectin undergoes little metabolism, which means that the environment will be exposed to a considerable amount of the active substance. The main route of excretion is faecal excretion, which accounts for 90% of the dose administered. Less than 2% is excreted in urine[24]. In several studies, the effect of anthelmintic drugs on different forms of life has been evaluated. In a study[12] in Switzerland the impact of a low concentration of ivermectin in dung was tested under field conditions. The study was conducted over the course of several years and several seasons. A significant reduction of flies (*Diptera*) and wasps (*Hymenoptera*) was found. The most important conclusion of this study was that the toxic non-target impact of ivermectin is considerable even at rather low substance concentrations in the dung[12]. In another study[10] the effect of several antiparasitic drugs from diverse groups against the common earthworm (*Lumbricus terrestris*) was tested under laboratory conditions. Ivermectin was picked to represent the macrocyclic lactones, fenbendazole to represent the benzimidazoles. It was found that although ivermectin had a relatively low effect on mortality (2.5%), the motility of the earthworms was significantly reduced after being exposed to the drug. Contrary to ivermectin, the effect of fenbendazole on mortality was high, with 55% of the earthworms not surviving the exposure to this drug, while the effect on motility was not significant.

2.2 Pyrethroids

Pyrethroids are synthetic derivatives of natural pyrethrins. This group, which includes drugs such as deltamethrin and cypermethrin, is mostly used against ectoparasites such as insects, lice and mites[25].

In comparison to the risks of resistant populations of endoparasites such as helminths, resistance in ectoparasites does not receive the same level of attention. This does not mean resistance is not present amongst these parasites, because it has been established that resistance to certain drugs is present as well. For example, in a study[26] in Ireland resistance amongst the suckling louse *Bovicola bovis* against deltamethrin on 24% of the farms included in the study was found. In another study[27], conducted in the UK, resistance amongst *B. bovis* to deltamethrin was found as well. In this study under laboratory conditions, it was demonstrated that even concentrations of 0.5% deltamethrin did not result in effective *B. bovis* mortality seeing that only after 180 minutes of exposure mortality was observed. At the end of the observation period of 1440 minutes, only a little over 60% mortality was achieved. This concentration of 0.5% deltamethrin is not only higher than the concentration present on the animal after treatment but also 10,000 times greater than the concentration considered to be enough to kill fully susceptible *B. bovis*. In the Netherlands, little research has been conducted regarding this topic and little information is therefore available on the current situation regarding resistance to pyrethroids in the Netherlands.

When applied as a pour-on formulation, 95% of deltamethrin has been shown to be excreted via the faeces. In this study[28], maximum concentration in the faeces was reached after 2 days and was still present at the end of the trial period of 8 days. These results showed that deltamethrin is rapidly absorbed via skin and elimination is slow, as deltamethrin was excreted for at least 8 days, most probably even longer. It has been reported that after a single dose of cypermethrin, concentrations were found in the faeces for up to 3 months[29].

In a study[25] conducted in Australia the toxicity of deltamethrin to dung beetle was assessed. The results of this study indicated that when cattle are treated with a formulation of deltamethrin, the residues are excreted in the faeces in quantities sufficient to have adverse effects on the development and/or survival of three different species of dung-breeding insects. This effect lasts for up to three weeks. Repeated treatment is confirmed to be a risk for dung beetles as well. It was indicated that a repeated treatment with deltamethrin every 3 weeks could lead to the extinction of the dung beetles in a given area.

2.3 Antiprotozoal drugs

Protozoal infections are treated with a variety of drugs. Coccidial infections can be treated with for example toltrazuril and diclazuril, both of these drugs belong to the chemical class of triazinetriones[30]. For both prevention and treatment of *Cryptosporidium parvum*, the drug halofuginone is commercially available[31].

While there are no reports about resistance to antiprotozoal drugs in cattle, there are reports on resistance to antiprotozoal drugs in other animal sectors and they suggest caution when using these drugs. In chickens, resistance amongst coccidia to various anticoccidial drugs has been established, including multi-drug resistant strains[32]. In small ruminants, drug resistance has been reported as well. In Norway, researchers found evidence that resistance to toltrazuril is present amongst species of ovine Eimeria. In 10% of the flocks a reduced efficacy was noted. This percentage is due to the inclusion criteria not representative for the true prevalence in Norway, however, it is clear that resistance is present[33]. Even more so, because another study confirmed the resistance to toltrazuril by evaluating the field isolate from the previously mentioned study. In this study[34] resistance was found amongst the most pathogenic Eimeria strain amongst sheep, *E. ovinoidalis*. This indicates that that severe clinical coccidiosis may be expected to occur in resistant flocks.

Pharmacokinetics vary extensively between different antiprotozoal drugs. According to the manufacturer, the bioavailability of halofuginone is 80%[31]. In contrast, diclazuril is absorbed only slightly[35]. This means that most of the dose is excreted unchanged. Toltrazuril is absorbed, albeit slowly. However, its main excretion route is via the faeces in the active metabolite toltrazuril sulfone[36], a synonym for ponazuril.
Antiprotozoal drugs have an effect on the environment as well. A study[37] assessing the influence of toltrazuril and its metabolites toltrazuril sulfoxide and ponazuril in chicken manure on soil found several effects. Firstly, it was established that toltrazuril was able to migrate from this manure to the soil. Ponazuril was even able to migrate from the soil to vegetables. The first negative effect that toltrazuril and its metabolites had in the soil was a negative effect on plant growth. In this study lettuce and radish were used, and it was shown that the presence of toltrazuril could significantly inhibit the germination and root elongation. The second negative effect was on the microbial activities of the soil. Soil enzyme activities are widely used as a biological/biochemical indicator of soil quality and the presence of toltrazuril reduced several enzyme activities.

To estimate to what extent the use of antiparasitic drugs has a negative effect on society in terms of for instance drug resistance and effects on soil life in the Netherlands, it is necessary to know how often and which antiparasitic drugs are administrated. Currently, this topic has not been researched by other studies. Therefore, this will be the first study to quantify the use of antiparasitic drugs in cattle in the Netherlands by translating sales data of a veterinary practice into defined daily doses per animal (DDDA).

3. Materials and methods

We obtained the number of dispensed packages for the year 2021 and 2022 of antiparasitic drugs from the Universitaire Landbouwhuisdieren Praktijk (ULP), located in Harmelen, the Netherlands to dairy cattle farms. A total of 273 farms is included in this study, which is 1.89% of the total of dairy cattle farms in the Netherlands[38]. For each of the 16 different antiparasitic drugs sold by the ULP we determined per package the target species, age category, and the kilograms of cattle that can be treated with that specific package size. Additionally, we determined a correction factor to correct for the duration of exposure. We derived this correction factor directly from the claimed duration of the effective treatment following one administration as provided in the Summary of Product Characteristics (SPC) or from the duration of action in the case of pour-on formulations. In case this information was not available, we calculated the duration of the treatment arbitrary by multiplying the half-life of the active component by two.
For every farm, the analysis of dispensed packages was performed per quarter each year and related to the registered animal population per farm in this quarter. With the information regarding the animal population, the population at risk of being treated could be determined. This population can be expressed in either the total biomass produced, the average mass of animals housed, or a combination of both[39]. Using the information regarding the animal population per farm, we chose to use as a proxy for the population at risk for treatment the average mass of animals housed. In the obtained information regarding the animal population per farm, the number of animals was split up in two categories: cattle over the age of 2 years and cattle under the age of 2 years. Information from the Central Bureau of Statistics of the Netherlands (CBS) was used to determine the distribution in the category cattle under the age of two between cattle aged <1 year and cattle aged 1-2 years[40]. This way, three weight categories were defined; cattle aged >2, cattle aged <2, and cattle aged <1. For each category, a mean weight was determined. The mean weight was determined by using the standardized body weight in kilograms as provided by the SDA[41] and with the distribution of animals per category as given by the CBS. For the mean weights per category, see table 1.
The term defined daily dose (DDD) was introduced by the World Health Organization to express human antimicrobial drug use. This term provides a number of individual days treated[42]. Based on this term, a similar definition was developed for veterinary products. In the Netherlands, the term defined daily doses per animal per farm ($DDDA_F$) is used to evaluate antimicrobial use at farm level. The $DDDA_F$ represents the number of days an average animal, on a specific farm, is treated with antimicrobials[41]. We chose to use this term to express the use of antiparasitic drugs as well. The number of $DDDA_F$ is the sum of treated kilograms with the medication dispensed over a year divided by the average number of kilograms of animals present on a farm. The sum of treatable kilograms is corrected for the possible extended effect of the drug by multiplying it by the corresponding correction factor.
In addition to the $DDDA_F$, we calculated the number of defined daily doses per animal in the age group for which the drug is authorized ($DDDA_S$), using the weights of these age groups. For drugs not authorized for cattle producing milk for human consumption, the $DDDA_S$ was calculated using the number of cattle aged <2 years present at the farm in the corresponding quarter. Because information regarding the number of calves present was not available, for antiprotozoal drugs the $DDDA_S$ was calculated using the number of cattle aged <1 year.

Table 1: Mean weights per age category.

Age category	Mean weight (kg)
Cattle > 2 years	600
Cattle < 2 years	337,63
Cattle < 1 year	209,91

4. Results

4.1 DDDA$_F$

The DDDA$_F$ was determined per year. In 2021, 182 farms (66.7%) of the farms applied antiparasitic drugs. In 2022, 175 farms (64.1%) applied antiparasitic drugs. Some farms used antiparasitic drugs in both years, and some only in one year. Over both years, 51 farms (18.7%) did not use any antiparasitic drugs. Over both years, the mean, median, 75th percentile, and standard deviation were determined, see table 2. In both years, the mean and median lie far apart. In figures 1 and 2, the distribution is made visible for respectively the year 2021 and 2022. Figure 1 shows that in 2021 one farm has a DDDA$_F$ between 150 and 155. Figure 2 shows a similar picture for the year 2022.

DDDA$_F$

	2021	**2022**
Mean	10.01	12.91
Median	0.35	0.25
75th Percentile	8.98	11.78
Standard deviation	21.01	25.11

Table 2: The mean, median, 75th percentile, and standard deviation of the DDDA$_F$ for the year 2021 and 2022.

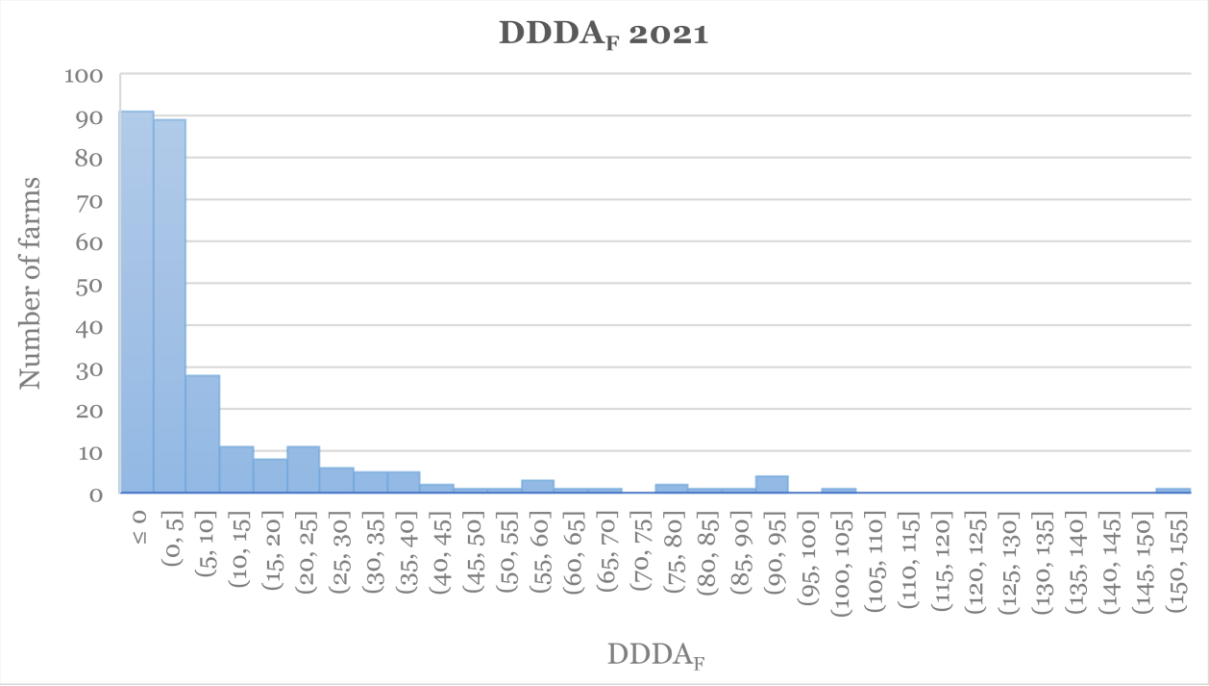

Figure 1: Histogram of the distribution of DDDA$_F$ for the year 2021.

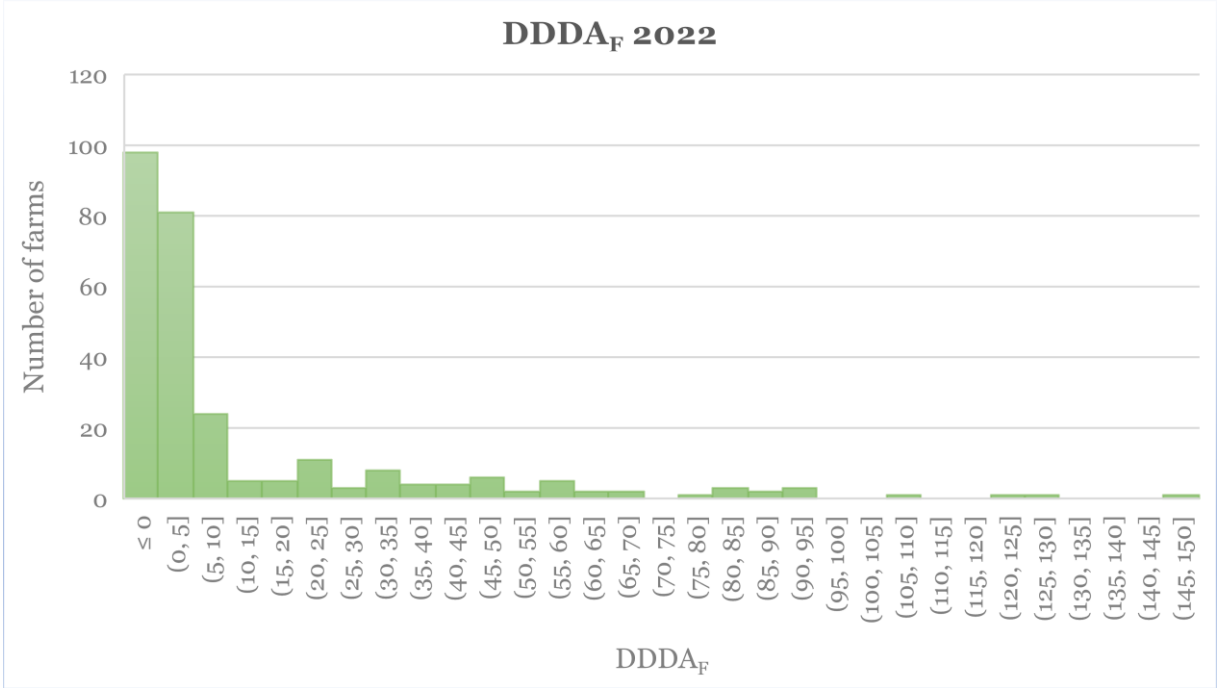

Figure 2: Histogram of the distribution of $DDDA_F$ for the year 2022.

4.2 DDDAs

The $DDDA_S$ are separated into two groups, specific for cattle aged <2 years and specific for cattle aged <1 year.

4.2.1. $DDDA_S$ 'Cattle aged <1'

The category 'Cattle aged <1' includes all antiprotozoal drugs, see table 3.

Brand name	**Active component**
Baycox Multi®	Toltrazuril
Cevazuril®	Toltrazuril
Halocur®	Halofuginone
Vecoxan®	Diclazuril

Table 3: Brand names and corresponding active components of all drugs included in the category 'Cattle aged <1', which includes all antiprotozoal drugs.

In 2021, 107 farms (39.2%) used drugs specifically authorized for calves. In 2022, 109 farms (39.9%) used drugs specifically authorized for calves. Some farms used drugs specifically authorized for calves in both years, and some only in one year. Over both years, 125 farms (45.8%) did not use any drugs specifically authorized for calves. Over both years, the mean, median, 75th percentile, and standard deviation were determined, see table 4. Because less than 50% of the farms applied these drugs, the median is 0. In figures 3 and 4, the distribution of the $DDDA_S$ 'Cattle aged <1 year' across the population is made visible for the year 2021 and 2022, respectively.

DDDAs 'Cattle aged <1 year'

	2021	2022
Mean	2.47	2.10
Median	0	0
75th Percentile	2.40	1.52
Standard deviation	7.50	4.49

Table 4: The mean, median, 75th percentile, and standard deviation of the DDDAs 'Cattle aged <1 year' for the year 2021 and 2022.

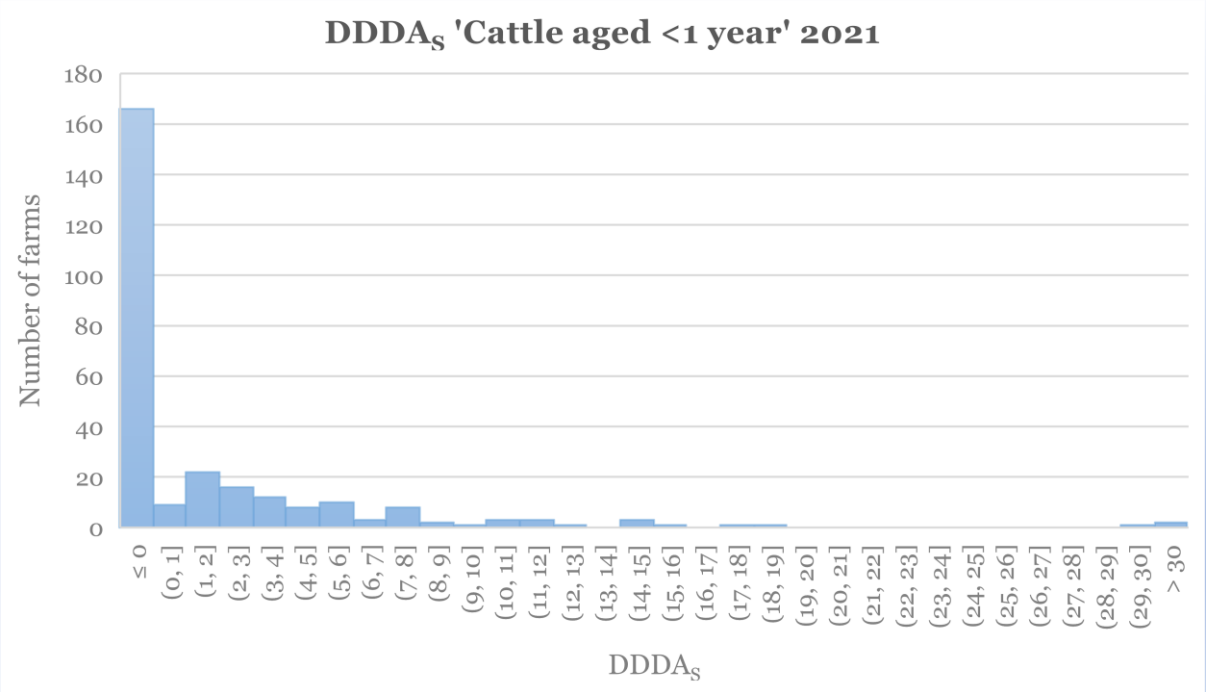

Figure 3: Histogram of the distribution of DDDAs 'Cattle aged <1 year' for the year 2021.

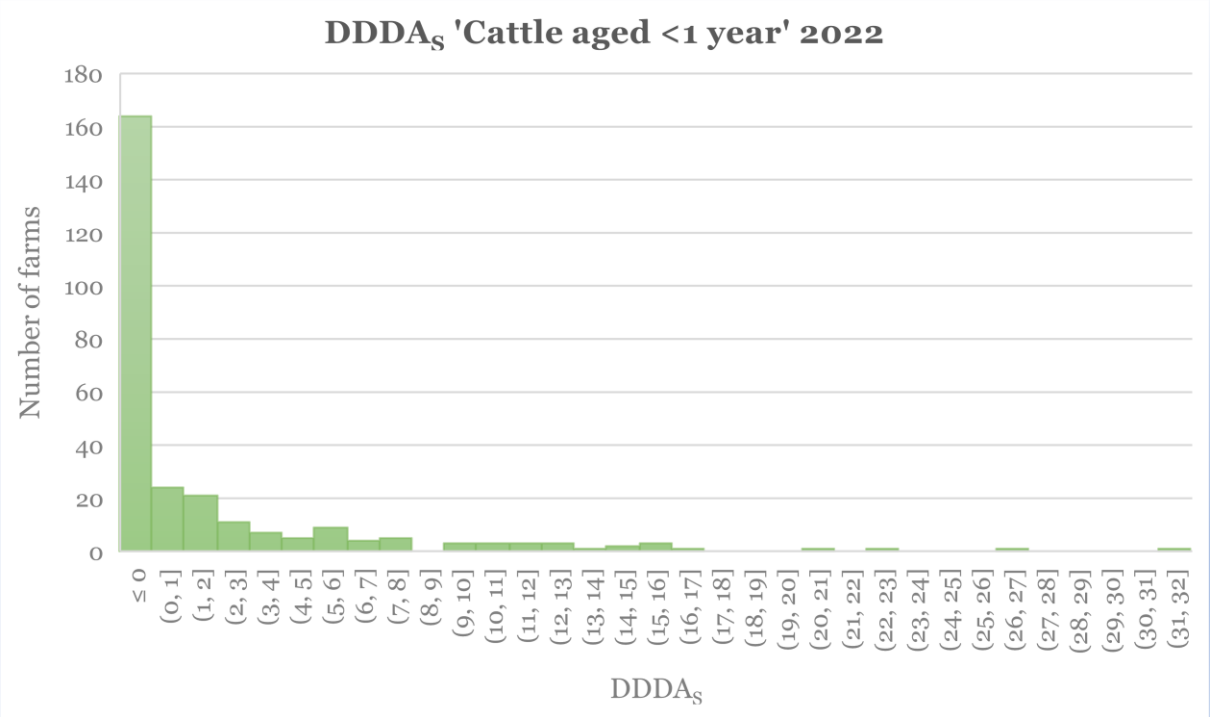

Figure 4: Histogram of the distribution of DDDA$_S$ 'Cattle aged <1 year' for the year 2021.

4.2.2 DDDAs 'Cattle aged <2 years'

Drugs that are not authorized for cattle producing milk for human consumption were put into the category 'Cattle aged <2 years', see table 5.

Brand name	**Active component**
Dectomax®	Doramectin
Ivomec®	Ivermectin
Noromectin®	Ivermectin
Repidose 5®	Oxfendazole
Repidose Forte®	Oxfendazole

Table 5: Brand names and corresponding active components of all drugs included in the category 'Cattle aged <2', which includes all drugs not authorized for cattle producing milk for human consumption.

In 2021, 60 farms (22.0%) used drugs specifically authorized for cattle aged <2 years. In 2022, 45 farms (16.5%) used drugs specifically authorized for cattle aged < 2 years. Some farms used drugs specifically authorized for cattle aged <2 years in both years, and some only in one year. Over both years, 194 farms (71.1%) did not use any drugs specifically authorized for cattle aged < 2 years. Over both years, the mean, median, 75th percentile, and standard deviation were determined, see table 6. Because less than 25% of the farms applied these drugs, both the median and 75th percentile are 0. In figures 5 and 6, the distribution of the DDDA$_S$ 'Cattle aged <2 years' across the population is made visible for the year 2021 and 2022, respectively.

DDDAs 'Cattle aged <2 years'

	2021	2022
Mean	10.47	9.10
Median	0	0
75th Percentile	0	0
Standard deviation	47.61	49.63

Table 6: The mean, median, 75th percentile, and standard deviation of the DDDAs 'Cattle aged <2 years' for the year 2021 and 2022.

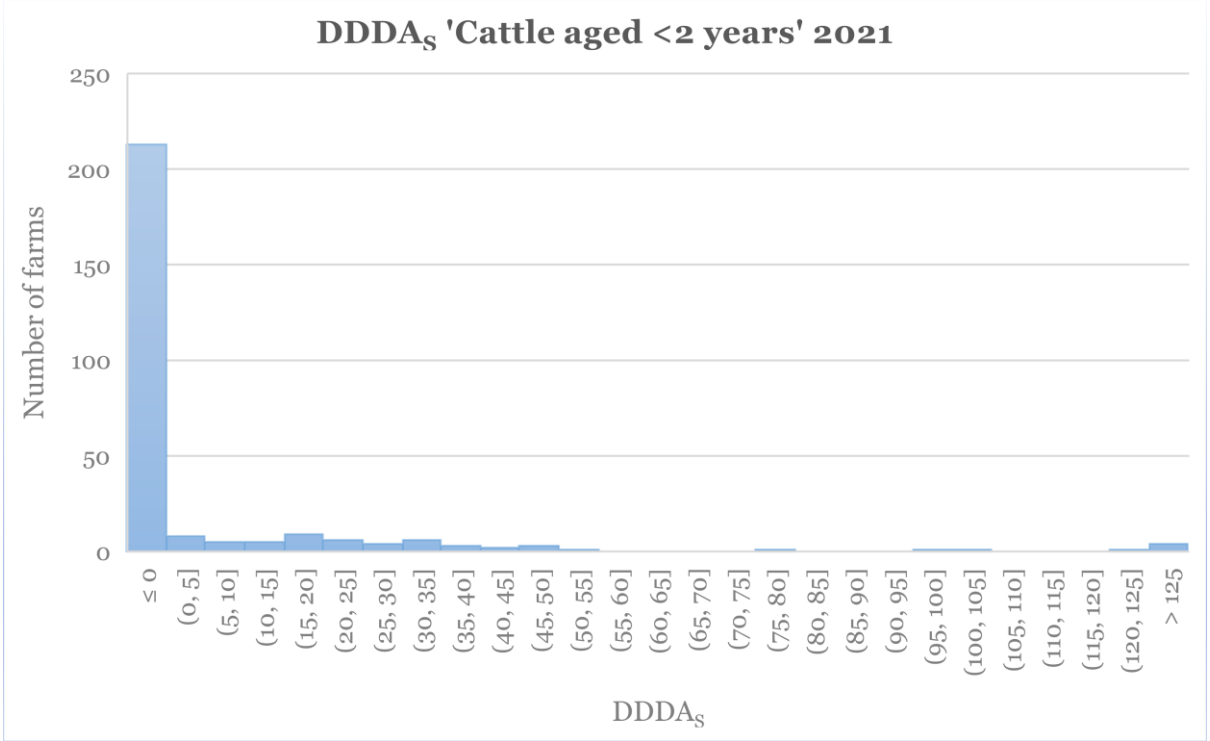

Figure 5: Histogram of the distribution of DDDAs 'Cattle aged <2 years' for the year 2021.

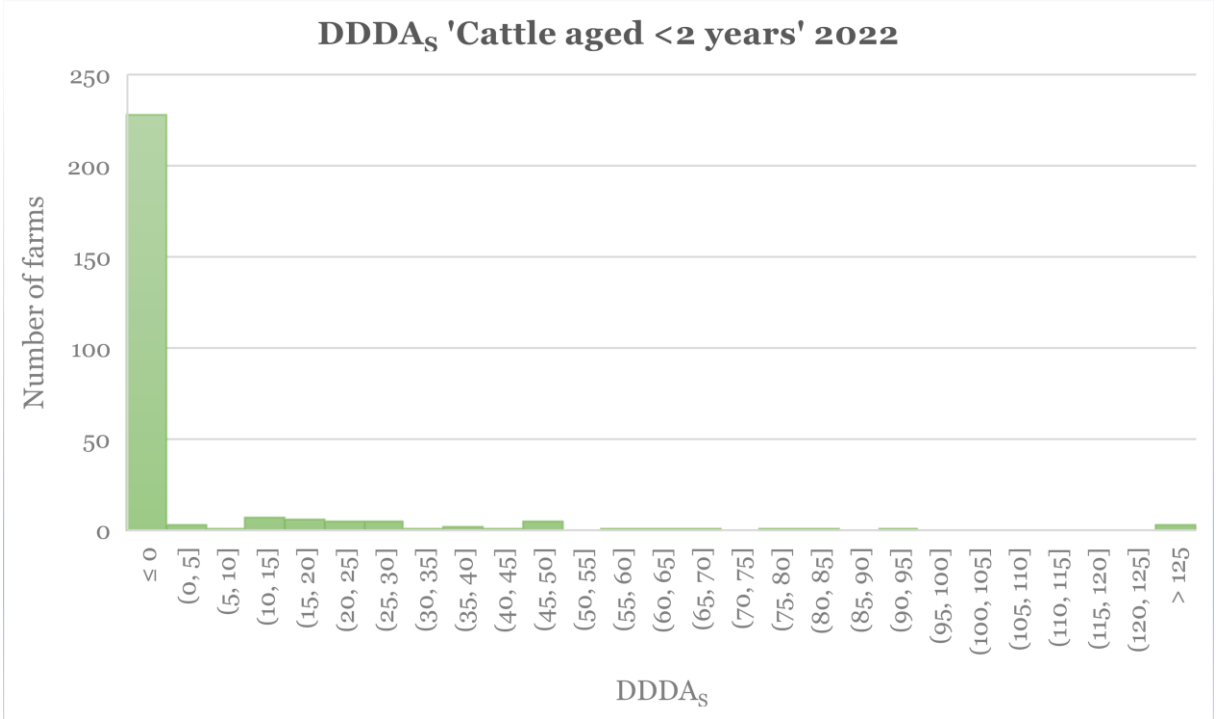

Figure 6: Histogram of the distribution of DDDA$_S$ 'Cattle aged <2 years' for the year 2022.

4.3 Distribution between pharmacotherapeutic groups

The antiparasitic drugs were divided into three groups, based on their ATCvet code. The three different groups that were identified are anthelmintic drugs, pyrethroids, and antiprotozoal drugs, see table 7. Each group consists of 2 to 6 different active components. The unit that was used to compare the different groups is the amount of treatable kilograms of animal corrected for extended effect. Table 8 shows that pyrethroids and anthelmintic drugs make up for far larger parts of the total of treatable kilograms of animal corrected for extended effect than antiprotozoal drugs. It is shown that the total of exposure based on sold VMP's converted to treatable kg's is 31,2% higher in 2022 than in 2021.

Anthelmintic drugs	**Pyrethroids**	**Antiprotozoal drugs**
Doramectin	Deltamethrin	Diclazuril
Eprinomectin	Permethrin	Halofuginone
Ivermectin		Toltrazuril
Oxfendazole		
Oxyclozanide		
Triclabendazole		

Table 7: The different active components divided between the three different pharmacotherapeutic groups, anthelmintic drugs, pyrethroids, and antiprotozoal drugs.

Distribution between groups

	2021	2022
Total of treatable kilograms of animal	187,279,201.60	245,644,089.60
Anthelmintic drugs (%)	39.2	29.4
Pyrethroids (%)	59.4	69.5
Antiprotozoal drugs (%)	1.4	1.1

Table 8: The distribution between the different pharmacotherapeutic groups defined in total of treatable kilograms of animal corrected for extended effect, for the year 2021 and 2022.

4.4 Distribution within pharmacotherapeutic group

4.4.1 Anthelmintic drugs

In 2021, 101 farms (37.0%) applied anthelmintic drugs. In 2022, 81 farms (29.7%) applied anthelmintic drugs. Some farms used anthelmintic drugs in both years, and some only in one year. Over both years, 143 farms (52.4%) did not use any anthelmintic drugs. Table 9 shows the distribution of the total of treatable kilograms of animal corrected for extended effect of anthelmintic drugs amongst the different active substances. It shows that eprinomectin, ivermectin and oxfendazole make up for the largest part, 98.47% and 98.58% for the year 2021 and 2022, respectively. For both years, the mean, median, 75th percentile, and standard deviation were determined, see table 10. The unit that was used to calculate these is $DDDA_F$. Because less than 50% of the farms applied anthelmintic drugs, the median is 0. In figures 7 and 8, the distribution of the $DDDA_F$ of anthelmintic drugs across the population is made visible for the year 2021 and 2022, respectively.

Distribution within anthelmintic drugs

	2021	2022
Total of treatable kilograms of animal	73,436,606.72	72,159,233.37
Doramectin (%)	0	0.003
Eprinomectin (%)	48.23	51.03
Ivermectin (%)	25.43	29.71
Oxfendazole (%)	24.81	17.84
Oxyclozanide (%)	0.06	0.02
Triclabendazole (%)	1.47	1.40

Table 9: The distribution of the total of treatable kilograms of animal with anthelmintic drugs corrected for extended effect between the different active components, for the year 2021 and 2022.

$DDDA_F$ anthelmintic drugs

	2021	2022
Mean	4.00	3.35
Median	0	0
75th Percentile	4.37	3.05
Standard deviation	9.40	9.23

Table 10: The mean, median, 75th percentile and standard deviation of the $DDDA_F$ of anthelmintic drugs for the year 2021 and 2022.

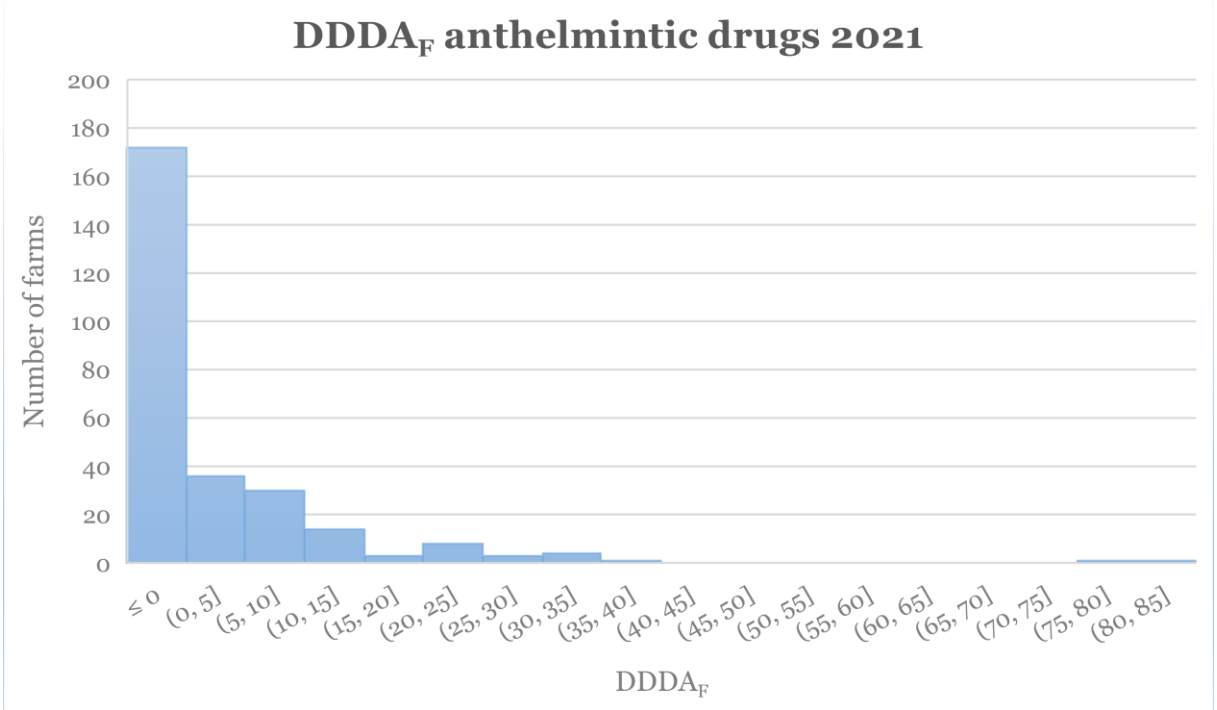

Figure 7: Histogram of the distribution of $DDDA_F$ of anthelmintic drugs for the year 2021.

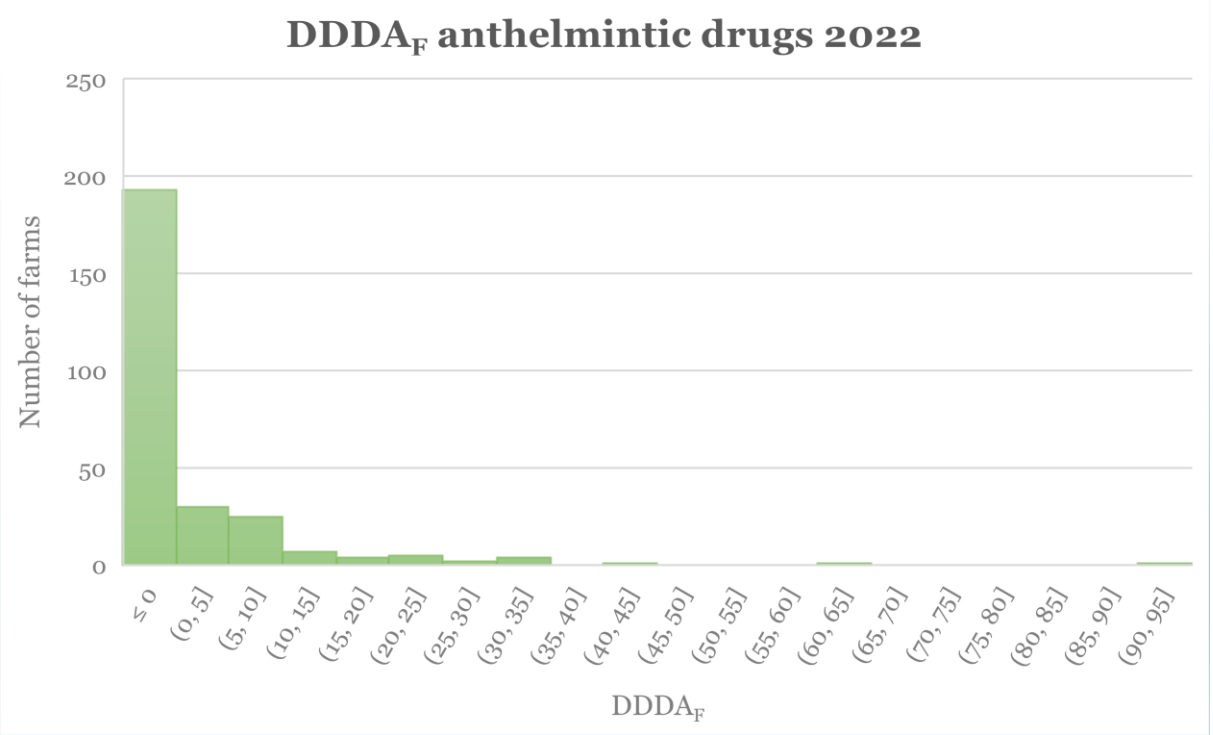

Figure 8: Histogram of the distribution of DDDA$_S$ of anthelmintic drugs for the year 2022.

4.4.2 Pyrethroids

In 2021, 35 farms (12.8%) applied pyrethroids. In 2022, 54 farms (19.7%) applied pyrethroids. Some farms used pyrethroids in both years, and some only in one year. Over both years, 206 farms (75.5%) did not use any pyrethroids. Table 11 shows the distribution of the total of treatable kilograms of animal corrected for extended effect of pyrethroids amongst the different active substances. It shows that deltamethrin makes up for the largest part, 82.78% and 95.74% for the year 2021 and 2022, respectively. For both years, the mean, median, 75th percentile, and standard deviation were determined, see table 12. The unit that was used to calculate these is DDDA$_F$. Because less than 25% of the farms applied pyrethroids, the median and 75th percentile are both 0. Figures 9 and 10 show the outliers in this population for both years. In both 2021 and 2022, one or more farms have a DDDA$_F$ value of more than 115.

Distribution within pyrethroids

	2021	**2022**
Total of treatable kilograms of animal	111,216,000	170,736,000
Deltamethrin (%)	82.78	95.74
Permethrin (%)	17.22	4.26

Table 11: The distribution of the total of treatable kilograms of animal with pyrethroids corrected for extended effect between the different active components, for the year 2021 and 2022.

$DDDA_F$ pyrethroids

	2021	2022
Mean	6.01	9.45
Median	0	0
75th Percentile	0	0
Standard deviation	19.50	23.47

Table 12: The mean, median, 75th percentile and standard deviation of the $DDDA_F$ of pyrethroids for the year 2021 and 2022.

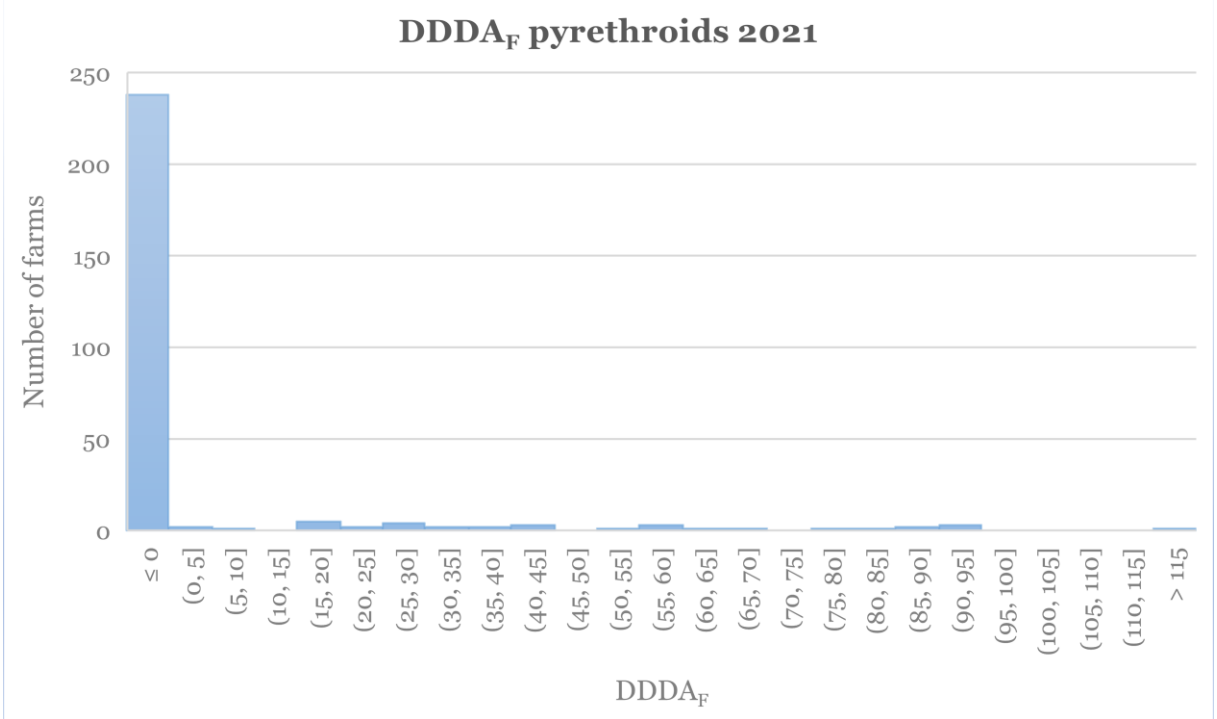

Figure 9: Histogram of the distribution of $DDDA_F$ of pyrethroids for the year 2021.

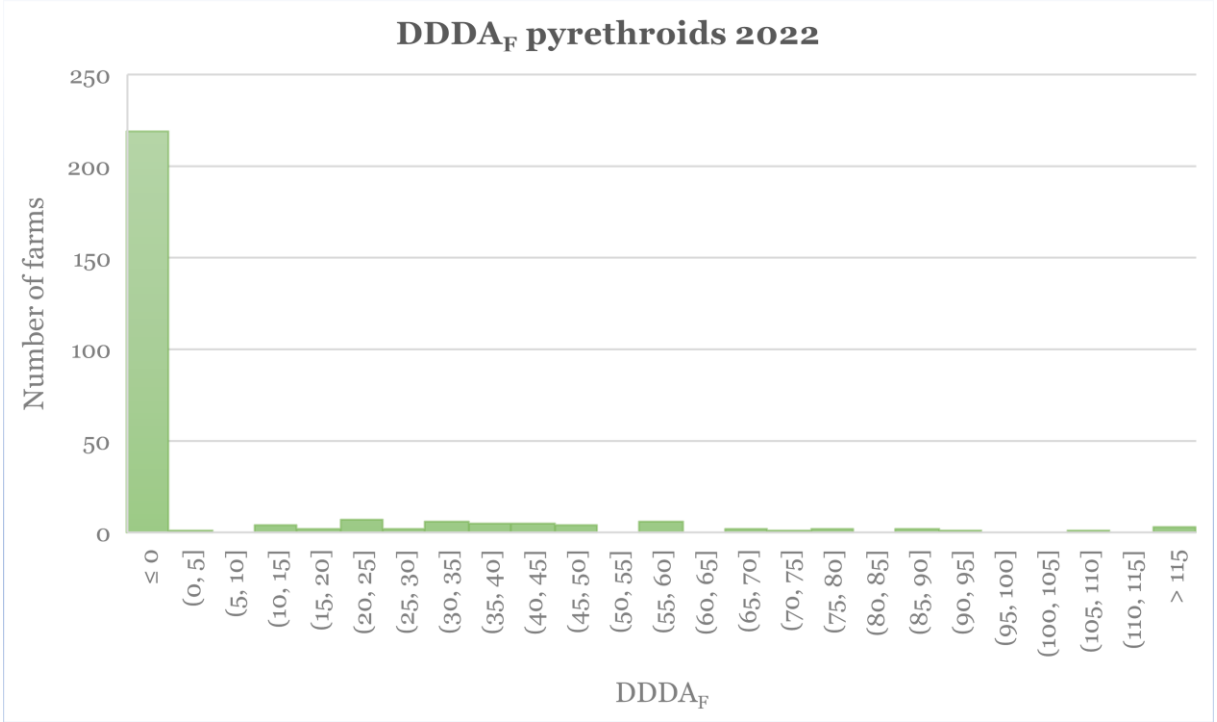

Figure 10: Histogram of the distribution of $DDDA_F$ of pyrethroids for the year 2022.

4.4.3 Antiprotozoal drugs

In 2021, 107 farms (39.2%) applied antiprotozoal drugs. In 2022, 109 farms (39.9%) applied antiprotozoal drugs. Some farms used antiprotozoal drugs in both years, and some only in one year. Over both years, 125 farms (45.8%) did not use any antiprotozoal drugs. Table 13 shows the distribution of the total of treatable kilograms of animal corrected for extended effect of antiprotozoal drugs amongst the different active substances. This table shows that halofuginone and toltrazuril together make up for the largest part, 98.33% and 98.66% for the year 2021 and 2022, respectively. In table 14, the mean, median, 75th percentile, and standard deviation are shown for the year 2021 and 2022. The unit that was used to calculate these is $DDDA_F$. Because less than 50% of the population applied antiprotozoal drugs, the median is zero. In figures 11 and 12, the distribution of the $DDDA_F$ of antiprotozoal drugs across the population is made visible for the year 2021 and 2022, respectively.

Distribution within antiprotozoal drugs

	2021	**2022**
Total of treatable kilograms of animal	2,626,594.91	2,748,856.24
Diclazuril (%)	1.67	1.34
Halofuginone (%)	56.09	61.39
Toltrazuril (%)	42.24	37.27

Table 13: The distribution of the total of treatable kilograms of animal with antiprotozoal drugs corrected for extended effect between the different active components, for the year 2021 and 2022.

DDDA$_F$ antiprotozoal drugs

	2021	2022
Mean	0.11	0.12
Median	0	0
75th Percentile	0.14	0.09
Standard deviation	0.24	0.24

Table 14: The mean, median, 75th percentile and standard deviation of the DDDA$_F$ of antiprotozoal drugs for the year 2021 and 2022.

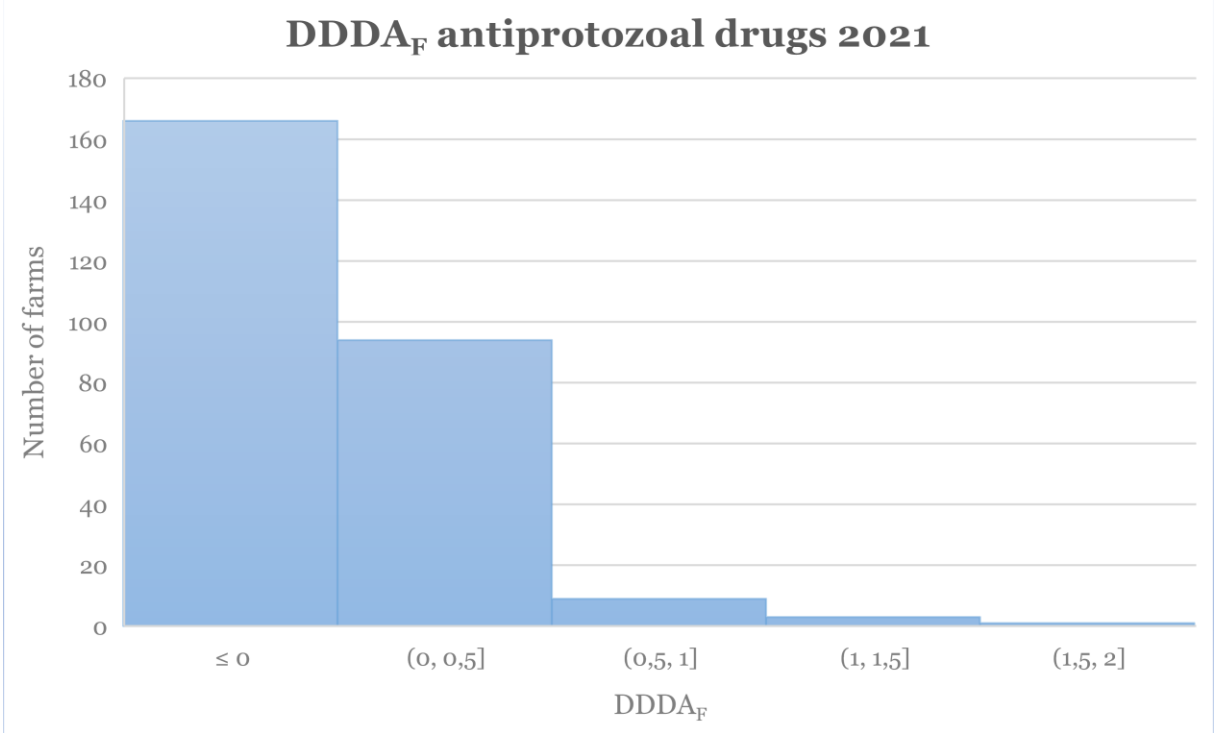

Figure 11: Histogram of the distribution of DDDA$_F$ of antiprotozoal drugs for the year 2021.

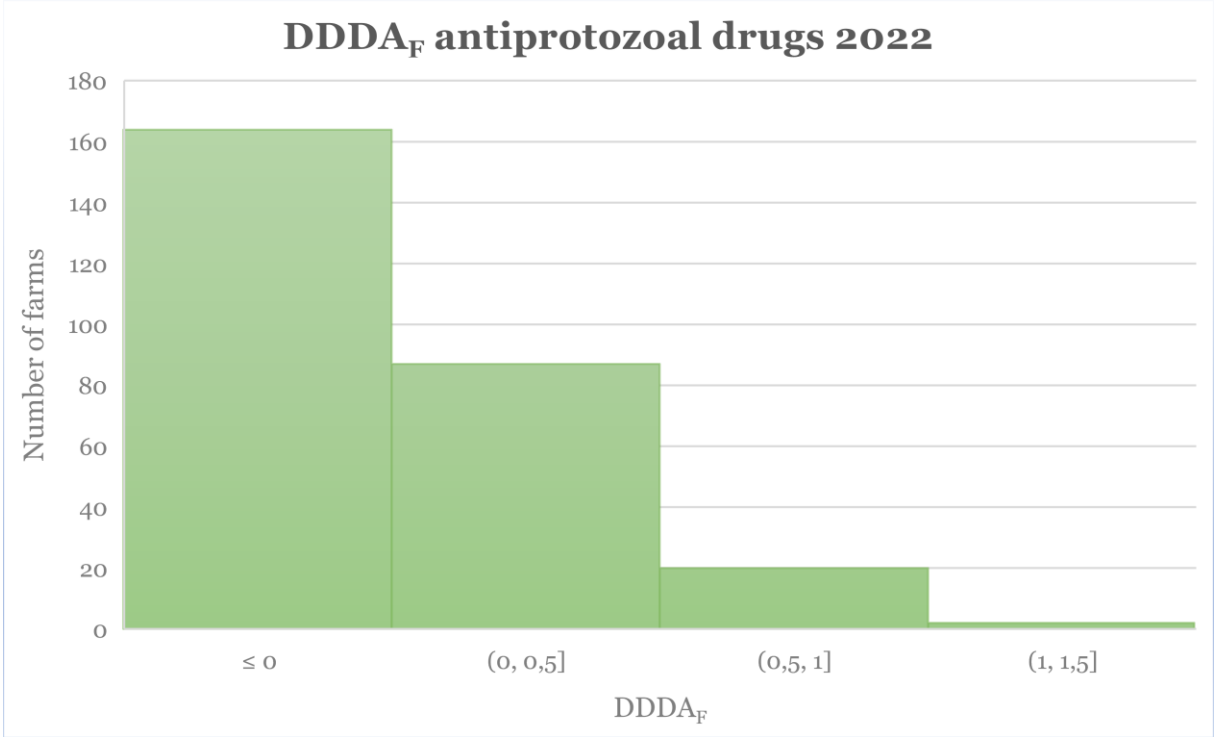

Figure 12: Histogram of the distribution of DDDA$_F$ of antiprotozoal drugs for the year 2021.

4.5 Seasonal effect

To determine whether there is a seasonal effect, the distribution of the DDDA is shown throughout the different quarters. This is determined for the DDDA$_F$, DDDA$_S$ 'Cattle aged <1 year', and DDDA$_S$ 'Cattle aged <2 year'.

4.5.1 Seasonal distribution DDDA$_F$

In figures 13 and 14, the total of DDDA$_F$ of all the farms included in this study are shown per quarter of the year 2021 and 2022, respectively. Per quarter is shown how this total is build up from the different active components. In the year 2021, the total of DDDA$_F$ of quarter 2 and 3 is 4.22 times greater than the total of DDDA$_F$ of quarter 1 and 4. For the year 2022, the total of DDDA$_F$ of quarter 2 and 3 is 4.15 times greater than the total of DDDA$_F$ of quarter 1 and 4. In both years, the pyrethroids, deltamethrin in particular, contributes for a great part to the total of DDDA$_F$. In the year 2021, the pyrethroids contribute for 72.51% of the total of DDDA$_F$ of quarter 2 and 3. In the year 2022, the pyrethroids contribute for 81.99% of the total of DDDA$_F$ of quarter 2 and 3.

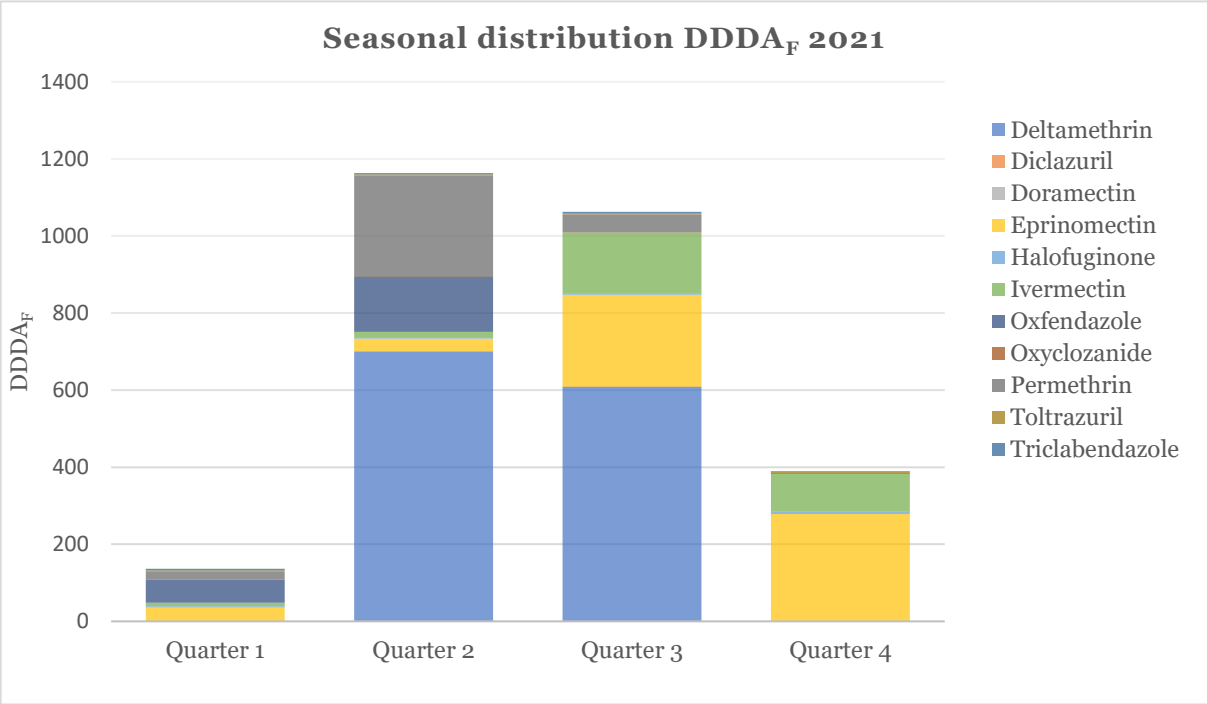

Figure 13: Histogram of the total of DDDA$_F$ of all the farms included in this study per quarter of the year 2021. Per quarter is shown how the total is build up from the different active components.

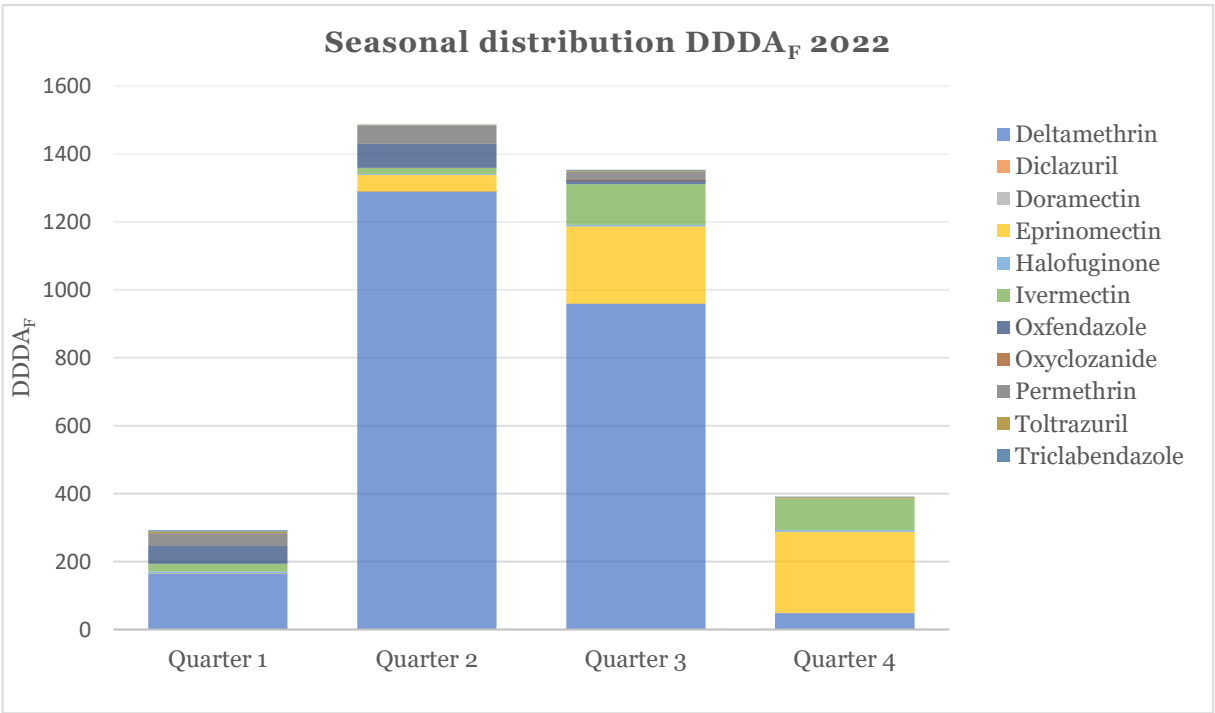

Figure 14: Histogram of the total of DDDA$_F$ of all the farms included in this study per quarter of the year 2022. Per quarter is shown how the total is build up from the different active components.

4.5.2 Seasonal effect DDDAs 'Cattle aged < 1 year'

In figures 15 and 16, the total of DDDAs 'Cattle aged <1 year' is shown per quarter for the year 2021 and 2022, respectively. Per quarter is shown how this total is build up from the different active components. In 2021, the total of DDDAs 'Cattle aged <1 year' for quarter 4 is 2.00 times greater than the mean of quarter 1, 2, and 3. In 2022, the total of DDDAs 'Cattle aged <1 year' of quarter 1 and 4 is 1.76 times greater than the total of DDDAs 'Cattle aged <1 year' of quarter 2 and 3.

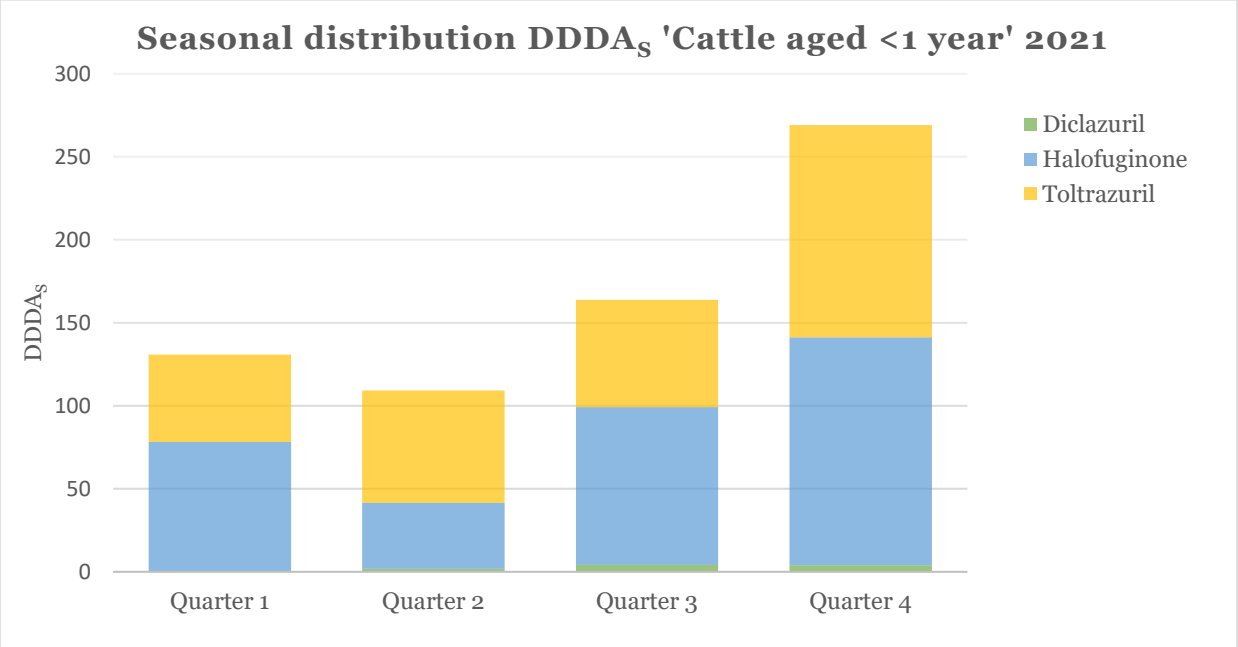

Figure 15: Histogram of the total of DDDAs 'Cattle aged <1 year' per quarter of the year 2022. Per quarter is shown how the total is build up from the different active components.

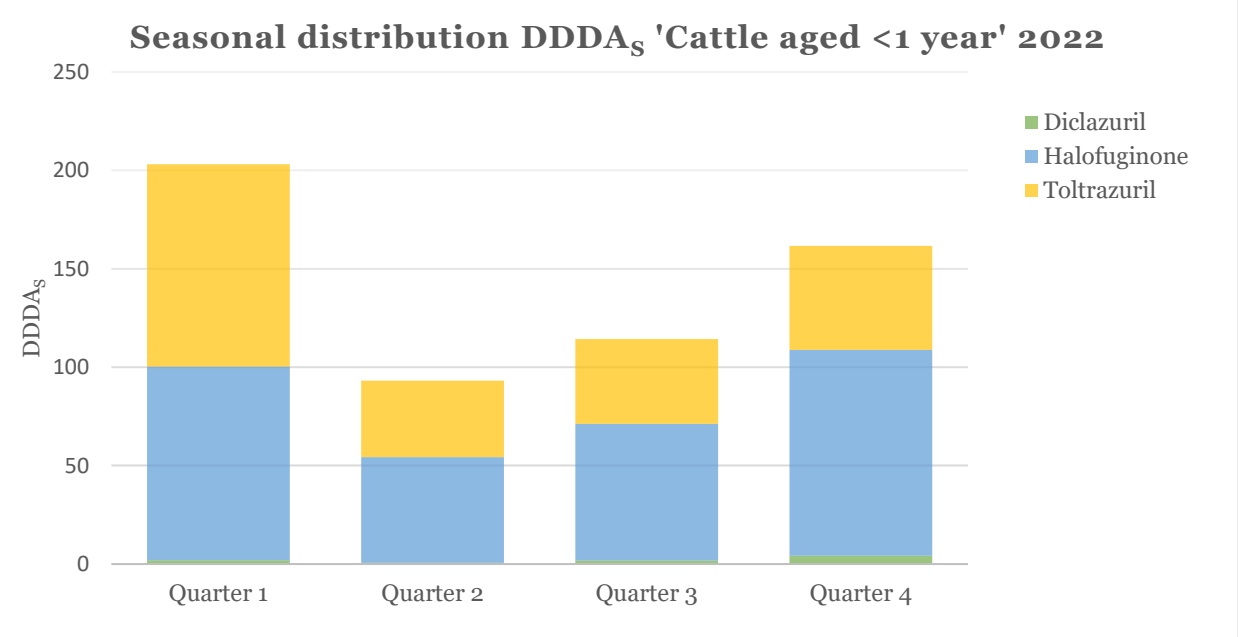

Figure 16: Histogram of the total of DDDAs 'Cattle aged <1 year' per quarter of the year 2021. Per quarter is shown how the total is build up from the different active components.

4.5.3 Seasonal effect DDDAs 'Cattle aged <2 years'

In figures 17 and 18, the total of DDDAs 'Cattle aged <2 years' is shown per quarter for the year 2021 and 2022, respectively. Per quarter is shown how this total is build up from the different active components. In 2021, the total of DDDAs 'Cattle aged <2 years' of quarter 2 and 3 is 1.82 times greater than the total of DDDAs 'Cattle aged <2 years' of quarter 1 and 4. In 2022, quarter 1 is the outlier, being 1.77 times greater than the mean of quarter 2, 3, and 4.

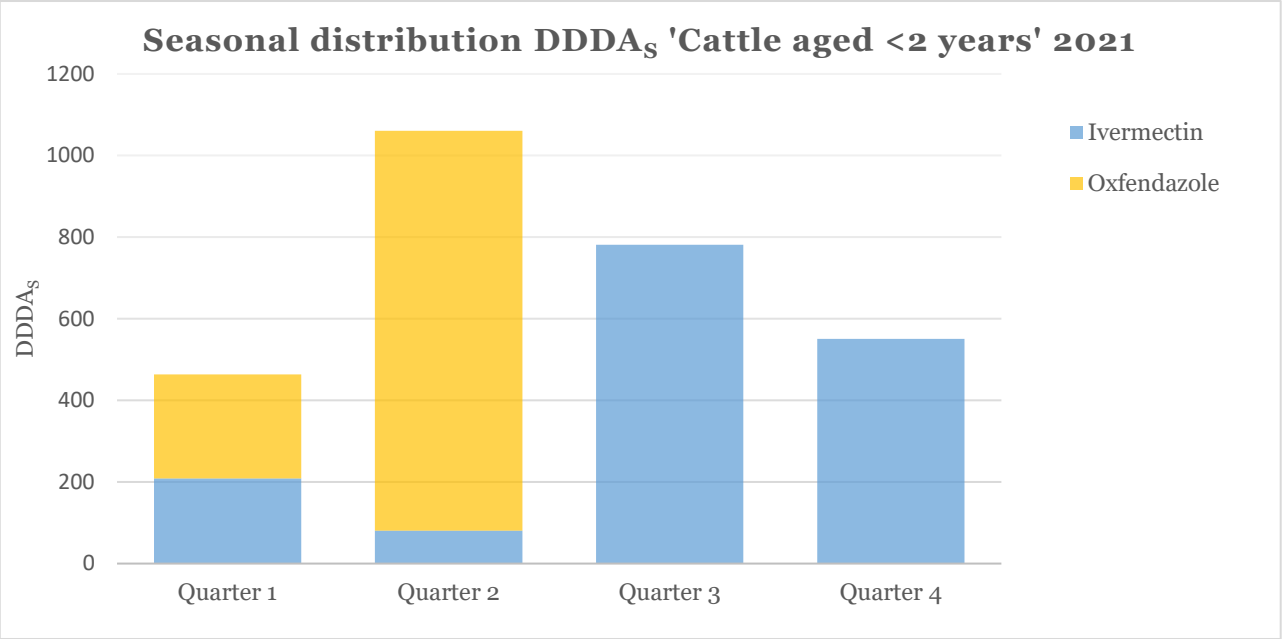

Figure 17: Histogram of the total of DDDAs 'Cattle aged <2 years' per quarter of the year 2021. Per quarter is shown how the total is build up from the different active components.

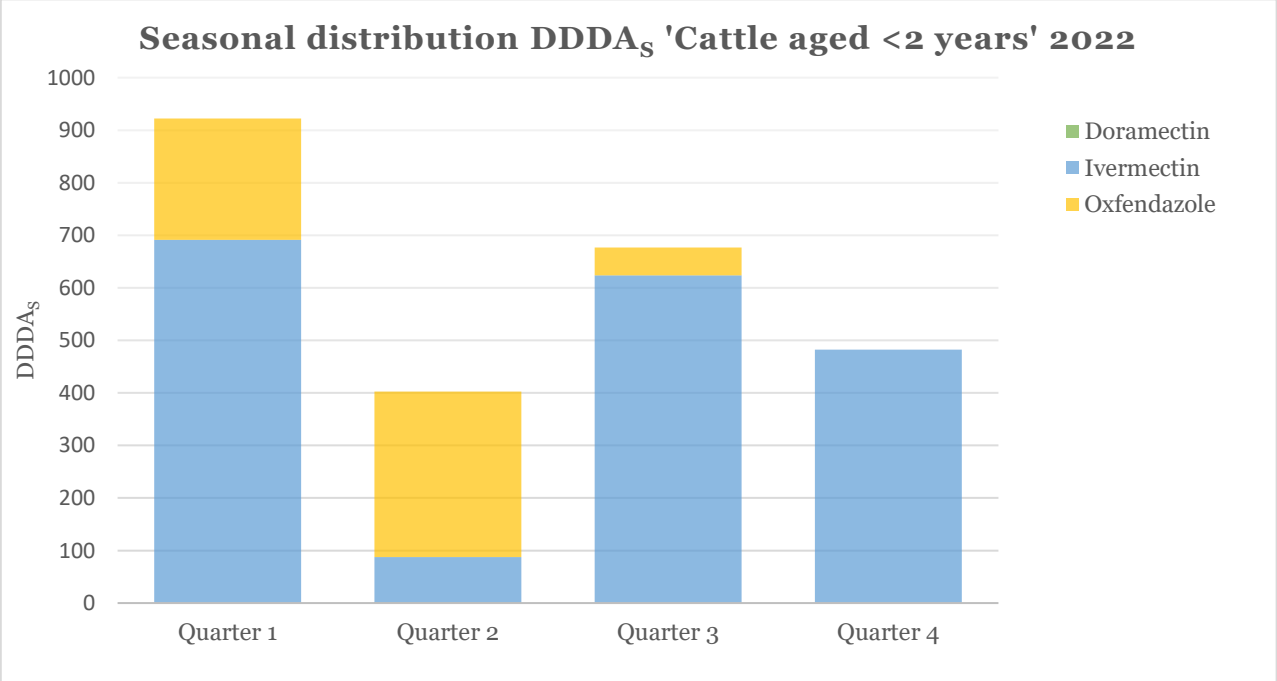

Figure 18: Histogram of the total of DDDAs 'Cattle aged <2 years' per quarter of the year 2022. Per quarter is shown how the total is build up from the different active components.

5. Discussion

In this study we established DDDA values for antiparasitic drugs and used that to calculate the number of $DDDA_F$ and $DDDA_S$ for antiparasitic drugs for dairy cattle based on dispensed VMP's in 2021 and 2022. When looking at the $DDDA_F$, it is clear that there is a large variation in the use of these drugs within the study population. In both years, the mean and median are very different, which indicates that the results are not correctly summarized by providing the mean. The mean $DDDA_F$ implies that the average cow on a farm in this study population would be exposed to antiparasitic drugs for 10.01 and 12.91 days in the years 2021 and 2022, respectively. The median, however, shows that on 50% of farms, the average animal is exposed to only 0.35 and 0.25 days in the year 2021 and 2022, respectively. The presence of outliers is confirmed by the histogram showing the distribution, as in both years only one or more farms have a $DDDA_F$ of 150. In addition to these outliers, the number of farms with zero antiparasitic drug use also contributes to the skewed distribution, illustrated by the fact that the 75th percentile is relatively close to the mean in both years. The number of farms with no antiparasitic drugs use is noteworthy. It shows that many farms seem to operate without applying antiparasitic drugs. This raises the question whether the other farms could change their management and reduce the use of antiparasitic drugs.

We calculated two different $DDDA_S$ values for specific age groups, 'Cattle aged <1 year' and 'Cattle aged <2 years'. It is important to note with these values that these values do not necessarily represent all antiparasitic drug use in these age categories as VMP's were assigned to the groups based on their label. Thus, these values only give an indication of the VMP's authorized for this specific age category. By assigning these VMP's to this age category and relating the treatable kg's to the corresponding estimated weight, this value represents the exposure of the study population to these drugs more truthfully compared to how these drugs are represented in the $DDDA_F$ value. The $DDDA_S$ 'Cattle aged <1 year' includes only all antiprotozoal active substances and not antiparasitic drugs that are also labelled for older animals. As this category thus includes a subset of the total of antiparasitic VMP's, the calculated $DDDA_S$ is relatively low. Because per year less than 50% of the included farms apply these VMP's, the median is zero and therefore it is not useful to compare the mean and median. The histogram shows that there again many outliers are present. In both years, several farms have a $DDDA_S$ 'Cattle aged <1 year' of more than 30. The $DDDA_S$ 'Cattle aged <2 years' includes all VMP's not authorized for cows producing milk for human consumption. These VMPs' are sold to a relatively small number of farms. As a result, not only the median but the 75th percentile is zero as well. The mean, however, is relatively high. This shows that the farms that are applying these VMP's in young animals, use these VMP's extensively.

The distribution between the different pharmacotherapeutic drugs shows that the total of treatable kilograms, corrected for extended effect, is the largest for the pyrethroids. Especially in the year 2022, as the increase of the total of treatable kilograms, corrected for extended effect, is mostly due to an increase of the pyrethroids. This increase corresponds with an increase of 54.3% of the number of farms applying these drugs. However, the total number of farms applying pyrethroids is in both years still relatively low. The large part of pyrethroids in the total of treatable kilograms of animal, corrected for extended effect, corresponds with the contribution of pyrethroids to the $DDDA_F$. In 2021 and 2022 it makes up for respectively 60.0% and 73.2% of the mean $DDDA_F$. The large part of pyrethroids is partly explained by the formulations of the pyrethroids included in this study. Of the two different drugs included in this category, one was in pour-on formulation and the other was formulated as an ear tag. These formulations have

a long-lasting effect, which results in extended exposure. Antiprotozoal drugs make up for the least part of the mean $DDDA_F$. This can partly be explained by the relatively high standardized animal weight in the age group compared to the actual weight of the treated animals. Also, the population at risk of being treated that is probably too high. Another aspect that contributes to the relative low total of DDDA of antiprotozoal drugs, is that the duration of effect of these drugs is relatively low. The antiprotozoal drugs included in this study are oral formulations with a correction factor ranging from 2.50 to 5.35 days. In comparison with other drugs in pour-on formulation or in bolus, these correction factors are low.

As this is the first study to calculate the DDDA values of antiparasitic drugs, it is not possible to compare the results with other studies. If or when other studies assess the use of antiparasitic drugs it would be interesting to eventually be able to benchmark this in a similar way antimicrobial use is currently benchmarked. In antimicrobial drug use, there is a clear trend of reduction in major livestock-producing countries in Europe. In the Netherlands for example, veterinary antimicrobial sales have declined with 70.8% in the period 2009-2021, with 2009 being the reference year chosen by the Dutch Government[43]. This shows that the monitoring and the measures taken were effective to reduce antimicrobial drug use. In the period 2011 to 2022, the European sales numbers of antiparasitic drugs have remained relatively stable[44]. This raises the question whether an approach with antiparasitic drugs similar to the approach to reduce antimicrobial drug use could yield similar results. This study could be start of this process.

For future studies, it would be better to divide the antiparasitic drugs into four categories. This study divided the antiparasitic drugs in antiprotozoal drugs, anthelmintic drugs, and pyrethroids. The macrocyclic lactones fall under the heading of anthelmintic drugs. However, contrary to other anthelmintic drugs, the macrocyclic lactones are effective against ectoparasites as well as endoparasites[13] and should therefore form their own category, the endectocides. This way it is more clear what part of the drugs is used for their effectiveness against endo- or ectoparasites, or both.

Defining antiparasitic drug use in DDDA has its limitations. The weight used to calculate the number of DDDA's is an estimated standard weight. When the actual weight at treatment is lower than the standard weight, a calculation based on the standard weight underestimates the antiparasitic drug use[42]. In this study, this is most likely the case with the calculation of the $DDDA_S$ of antiprotozoal drugs. These drugs are mostly used amongst calves with some drugs even having a weight limit of 80 kg. However, the obtained information regarding the animal population was not differentiated into the number of calves present on the farm and thus the $DDDA_S$ of drugs specific for calves was calculated using the standard weight and number of cattle aged <1. Another limitation is found in the dosage. For some VMP's, the dosage differs for different indications. For these VMP's the mean of the different dosages is calculated. When the distribution of the use of this drug is not distributed equally between the different indications, it is possible to over or underestimate the use of this drug.

The packaging size is in some case of great importance when calculating the DDDA. Some VMP's are commercially only available in large pack. The consequence of this, is that small farms will generate high DDDA values when such pack is dispensed to them. In the meantime, it is known that this pack might be used for several years. In this study, several farms that used anti-parasitic drugs in one year, seemingly used none in the other year because none were dispensed to them. This suggests that provided VMP's might be used for several years. This blurs the image of a DDDA value based on one year. Another risk of relatively large pack sizes is that the pack might not be used fully and is discarded. This

way, the true exposure of the population is misjudged when using the full packaging to calculate the DDDA, while it may also have environmental consequences. In both cases, whether it is used for several years or not used completely, a smaller pack size would be the better option and therefore it would be useful if pharmaceutical companies would put smaller pack sizes on the market.

By visualisation of the distribution of $DDDA_F$ and $DDDA_S$ over quarters of the years, a seasonal effect may be detected. In the distribution of $DDDA_F$ per quarter, it is clearly visible that the $DDDA_F$ value of quarter 2 and 3 is much higher than the $DDDA_F$ value of quarter 1 and 4. This indicates that there is a seasonal effect. In summer and spring, the seasons in which many dairy farms graze their animals, the use of antiparasitic drugs is higher than in winter and autumn, the seasons in which the largest part of the animal population is held indoors. It is shown that a large part of the $DDDA_F$ in quarter 2 and 3 is due to the use of pyrethroids. This would support the hypothesis that during grazing the use of antiparasitic drugs is higher, as these drugs are authorized for the use against (biting) flies[45;46]. The distribution of the $DDDA_S$ 'Cattle aged <1 year' shows the opposite. In 2021 the $DDDA_S$ 'Cattle aged <1 year' value is highest in quarter 4, in 2022 quarter 1 and 4 have the highest $DDDA_S$ 'Cattle aged <1 year' value. As this category includes all antiprotozoal drugs, these results suggest that protozoic infections are more common in autumn and winter. However, this is contrary to the results of a study by Trotz-Williams[47] in which it was found that the risk of *Cryptosporidium parvum* infections was significantly higher in summer months. The uncertainty of determining a seasonal effect by analysing the date on which the drug is provided, is that it cannot be known for sure that the package is used in the same period of time, as mentioned earlier.

More information about the study population would provide context for the calculated number of daily doses. For example, whether or not a farm applies grazing in its management is of great importance to know to what extent the animals are exposed to parasites. In addition to the grazing management of the farm, it would also be interesting to know more about the strategies the farmers apply when it comes to antiparasitic drugs. Strategies to reduce for example the use of anthelmintic drugs exist and have been studied. This includes targeted treatment (TT), where the whole flock/herd is treated based on the knowledge of the risk and targeted selected treatment (TST), where only individual animals within the grazing group are treated. In a review by Charlier et al.[48] it was concluded that these strategies are applicable at farm level and result in reduced costs of anthelmintic drugs, minimal risk to production objectives and improved sustainability of control. However, in a recent study[49] France it is shown that amongst sheep farmers low-use strategy is little applied when using anthelmintic drugs. Coprological analysis is an important part of the low-use strategy, and several farmers expressed their scepticism about the method of coprological analysis. The benefits of coprological analysis did not outweigh the extra costs, labour, time, and the loss of autonomy. In addition to this, farmers described preventive use as effective, habitual, and convenient. By studying the use of antiparasitic drugs amongst dairy cattle farmers in the Netherlands in more detail it could become clear to what extent low-use strategies in antiparasitic drug use are applied, which helps to devise measures to minimize antiparasitic drug use.

6. Conclusion

Because of the effects antiparasitic drugs have on the environment and the risk of drug resistance amongst parasites, it is preferable to apply antiparasitic drugs as little as possible without compromising on animal health. This study gained insight in the current use of antiparasitic drugs in dairy cattle in the Netherlands by calculating the number of DDDA's on farm level in 273 farms, 1.89% of the total of dairy cattle farms in the Netherlands, and on the level of drugs authorized for specific age categories. By analysing per quarter, the seasonal distribution has been determined. An indication of a seasonal effect in the use of antiparasitic drugs was found, however, to confirm this effect it should be studied more extensively. In the use of antiparasitic drugs, there is a wide spread. A large part of the population does not even use antiparasitic drugs at all. The wide spread together with the number of no use farms indicates that an overall reduction is possible, a reduction with the goal of preserving the ability to treat infected animals and minimize the effects for the environment.

7. Acknowledgements

I would like to thank everyone involved in this study, in particular Dr. T. van Werven, Dr. R. Jorritsma, and Dr. I.M. van Geijlswijk for their supervision during this project, their enthusiasm was contagious. In addition to this, I would like to thank Dr. T. van Werven for making the sales data of the ULP available.

8. References

1. Taylor MA, Coop RL, Wall RL. Parasites of Cattle. In: *Veterinary Parasitology*. 3rd ed. Blackwell Publishing; 2007:51-151.
2. Deplazes P, Eckert J, Mathis A, von Samson-Himmelstjerna G, Zahner H. Suborders Amblycera and Ischnocera (chewing lice). In: *Parasitology in Veterinary Medicine*. 1st ed. Wageningen Academic Publishers; 2016:450-453.
3. Verwoerd DW, Erasmus B. Bluetongue. In: Coetzer JAW, Tustin R. ., eds. *Infectious Diseases of Livestock*. 2nd ed. Oxford University Press; 2004:1201-1220.
4. Charlier J, van der Voort M, Kenyon F, Skuce P, Vercruysse J. Chasing helminths and their economic impact on farmed ruminants. *Trends Parasitol*. 2014;30(7):361-367.
5. Eysker M, Van Der Aar WM, Boersema JH, Githiori JB, Kooyman FNJ. The effect of repeated moves to clean pasture on the build up of gastrointestinal nematode infections in calves. *Vet Parasitol*. 1998;76(1-2):81-94. doi:10.1016/S0304-4017(97)00211-2
6. Claerebout E, Geldhof P. Helminth Vaccines in Ruminants: From Development to Application. *Vet Clin North Am - Food Anim Pract*. 2020;36(1):159-171. doi:10.1016/j.cvfa.2019.10.001
7. Sutherland IA, Leathwick DM. Anthelmintic resistance in nematode parasites of cattle: A global issue? *Trends Parasitol*. 2011;27(4):176-181. doi:10.1016/j.pt.2010.11.008
8. Köhler P. The biochemical basis of anthelmintic action and resistance. *Int J Parasitol*. 2001;31(4):336-345. doi:10.1016/S0020-7519(01)00131-X
9. Ellse L, Burden F, Wall R. Pyrethroid tolerance in the chewing louse Bovicola (Werneckiella) ocellatus. *Vet Parasitol*. 2012;188(1-2):134-139. doi:10.1016/j.vetpar.2012.03.018
10. Goodenough AE, Webb JC, Yardley J. Environmentally-realistic concentrations of anthelmintic drugs affect survival and motility in the cosmopolitan earthworm Lumbricus terrestris (Linnaeus, 1758). *Appl Soil Ecol*. 2019;137(January):87-95. doi:10.1016/j.apsoil.2019.02.001
11. Floate KD, Spooner RW, Colwell DD. Larvicidal activity of endectocides against pest flies in the dung of treated cattle. *Med Vet Entomol*. 2001;15(1):117-120. doi:10.1046/j.1365-2915.2001.00269.x
12. Jochmann R, Blanckenhorn WU. Non-target effects of ivermectin on trophic groups of the cow dung insect community replicated across an agricultural landscape. *Basic Appl Ecol*. 2016;17(4):291-299.
13. Floate KD. Endectocide use in cattle and fecal residues: Environmental effects in Canada. *Can J Vet Res*. 2006;70(1):1-10.
14. Adler N, Bachmann J, Blanckenhorn WU, Floate KD, Jensen J, Römbke J. Effects of ivermectin application on the diversity and function of dung and soil fauna: Regulatory and scientific background information. *Environ Toxicol Chem*. 2016;35(8):1914-1923. doi:10.1002/etc.3308
15. Mahefarisoa KL, Simon Delso N, Zaninotto V, Colin ME, Bonmatin JM. The threat of veterinary medicinal products and biocides on pollinators: A One Health perspective. *One Heal*. 2021;12:100237. doi:10.1016/j.onehlt.2021.100237

16. Belzunces LP, Tchamitchian S, Brunet JL. Neural effects of insecticides in the honey bee. *Apidologie*. 2012;43(3):348-370. doi:10.1007/s13592-012-0134-0

17. El Hassani AK, Giurfa M, Gauthier M, Armengaud C. Inhibitory neurotransmission and olfactory memory in honeybees. *Neurobiol Learn Mem*. 2008;90(4):589-595. doi:10.1016/j.nlm.2008.07.018

18. Rose Vineer H, Morgan ER, Hertzberg H, et al. Increasing importance of anthelmintic resistance in European livestock: Creation and meta-analysis of an open database. *Parasite*. 2020;27. doi:10.1051/parasite/2020062

19. Rose H, Rinaldi L, Bosco A, et al. Widespread anthelmintic resistance in European farmed ruminants: A systematic review. *Vet Rec*. 2015;176(21):546. doi:10.1136/vr.102982

20. Ploeger HW, Everts RR. Alarming levels of anthelmintic resistance against gastrointestinal nematodes in sheep in the Netherlands. *Vet Parasitol*. 2018;262(September):11-15. doi:10.1016/j.vetpar.2018.09.007

21. Moll L, Gaasenbeek CPH, Vellema P, Borgsteede FHM. Resistance of Fasciola hepatica against triclabendazole in cattle and sheep in The Netherlands. *Vet Parasitol*. 2000;91(1-2):153-158. doi:10.1016/S0304-4017(00)00267-3

22. Holzhauer M, Van Doorn DCK, Bartels CJM, et al. Wormmanagement en resistentie ontwikkeling op Nederlandse rundvee bedrijven. *Tijdschr Diergeneeskd*. 2014;2014-June(6):27-35.

23. Horvat AJ., Babić S, Pavlović D, et al. Analysis, occurrence and fate of anthelmintics and their transformation products in the environment. *TrAC Trends Anal Chem*. 2012;31(January):61-84.

24. González Canga A, Sahagún Prieto AM, José Diez Liébana M, Martínez NF, Vega MS, Vieitez JJG. The pharmacokinetics and metabolism of ivermectin in domestic animal species. *Vet J*. 2009;179(1):25-37. doi:10.1016/j.tvjl.2007.07.011

25. Wardhaugh KG, Longstaff BC, Lacey MJ. Effects of residues of deltamethrin in cattle faeces on the development and survival of three species of dung-breeding insect. *Aust Vet J*. 1998;76(4):273-280. doi:10.1111/j.1751-0813.1998.tb10159.x

26. Mckiernan F, O'Connor J, Minchin W, et al. A pilot study on the prevalence of lice in Irish beef cattle and the first Irish report of deltamethrin tolerance in Bovicola bovis. *Ir Vet J*. 2021;74(1):1-8. doi:10.1186/s13620-021-00198-y

27. Sands B, Ellse L, Mitchell S, Sargison ND, Wall R. First report of deltamethrin tolerance in the cattle chewing louse Bovicola bovis in the UK. *Vet Rec*. 2015;176(9):3p. doi:10.1136/vr.102777

28. Venant A, Belli P, Borrel S, Mallet J. Excretion of deltamethrin in lactating dairy cows. *Food Addit Contam*. 1990;7(4):347-356. doi:10.1080/02652039009373916

29. Virlouvet G, Bichon E, André F, Bizec B Le. Faecal elimination of cypermethrin by cows after pour-on administration: Determining concentrations and measuring the impact on dung beetles. *Toxicol Environ Chem*. 2006;88(3):489-499. doi:10.1080/02772240600703643

30. Stewart CG, Penzhorn BL. Coccidiosis. In: Coetzer JAW, Tustin RC, eds. *Infectious Diseases of Livestock*. 2nd ed. Oxford University Press; 2004:319-329.

31. EMA. Bijlage I samenvatting van de productkenmerken. Published online 2019:1-24. https://www.ema.europa.eu/en/documents/product-information/halocur-epar-product-information_nl.pdf

32. Lan LH, Sun BB, Zuo BXZ, Chen XQ, Du AF. Prevalence and drug resistance of avian Eimeria species in broiler chicken farms of Zhejiang province, China. *Poult Sci*. 2017;96(7):2104-2109. doi:10.3382/ps/pew499

33. Odden A, Denwood MJ, Stuen S, et al. Field evaluation of anticoccidial efficacy: A novel approach demonstrates reduced efficacy of toltrazuril against ovine Eimeria spp. in Norway. *Int J Parasitol Drugs Drug Resist*. 2018;8(2):304-311. doi:10.1016/j.ijpddr.2018.05.002

34. Odden A, Enemark HL, Ruiz A, et al. Controlled efficacy trial confirming toltrazuril resistance in a field isolate of ovine Eimeria spp. *Parasites and Vectors*. 2018;11(1):1-11. doi:10.1186/s13071-018-2976-4

35. Ministerie van LNV. SAMENVATTING VAN DE PRODUCTKENMERKEN. Published online 2020. https://db.cbg-meb.nl/marketedauth/v9660-90wmah-31082020.pdf

36. Ministerie van LNV. SAMENVATTING VAN DE PRODUCTKENMERKEN. Published online 2021. https://db.cbg-meb.nl/marketedauth/v118580-90vr-01112021.pdf

37. Huo M, Ma W, Zhou K, Xu X, Liu Z, Huang L. Migration and toxicity of toltrazuril and its main metabolites in the environment. *Chemosphere*. 2022;302.

38. SDA. Het gebruik van antibiotica bij landbouwhuisdieren in 2022. Published 2022. Accessed January 22, 2024. https://cdn.i-pulse.nl/autoriteitdiergeneesmiddelen/userfiles/sda jaarrapporten ab-gebruik/AB-rapport 2022/def-sda-rapport-met-brief---het-gebruik-van-antibiotica-bij-landbouwhuisdieren-in-2022-erratum20230912(1).pdf

39. Sanders P, Vanderhaeghen W, Fertner M, et al. Monitoring of Farm-Level Antimicrobial Use to Guide Stewardship: Overview of Existing Systems and Analysis of Key Components and Processes. *Front Vet Sci*. 2020;7(August). doi:10.3389/fvets.2020.00540

40. CBS. Landbouw; gewassen, dieren, grondgebruik en arbeid op nationaal niveau. Published 2023. https://opendata.cbs.nl/#/CBS/nl/dataset/81302ned/table?searchKeywords=melkvee

41. SDA. Standard operating procedure. Published online 2020:1-13. https://cdn.i-pulse.nl/autoriteitdiergeneesmiddelen/userfiles/overige rapporten/sop-rekensystematiek-website-03032020-1(1).pdf

42. Collineau L, Belloc C, Stärk KDC, et al. Guidance on the Selection of Appropriate Indicators for Quantification of Antimicrobial Usage in Humans and Animals. *Zoonoses Public Health*. 2017;64(3):165-184. doi:10.1111/zph.12298

43. SWAB. NethMap 2022. Published 2022. Accessed January 23, 2024. https://swab.nl/nl/exec/file/download/197

44. AnimalhealthEurope. The European animal medicines industry in figures. Report. Published 2022. Accessed January 10, 2024. https://animalhealtheurope.eu/facts-and-figures/

45. Ministerie van LNV. SAMENVATTING VAN DE PRODUCTKENMERKEN. Published online 2014. https://db.cbg-meb.nl/marketedauth/v9330-90wr-03122014.pdf

46. Ministerie van LNV. SAMENVATTING VAN DE PRODUCTKENMERKEN. Published online 2016. https://db.cbg-meb.nl/marketedauth/v104958-90wm-09092016.pdf

47. Trotz-Williams LA, Wayne Martin S, Leslie KE, Duffield T, Nydam D V., Peregrine AS. Calf-level risk factors for neonatal diarrhea and shedding of Cryptosporidium parvum in Ontario dairy calves. *Prev Vet Med*. 2007;82(1-2):12-28. doi:10.1016/j.prevetmed.2007.05.003

48. Charlier J, Morgan ER, Rinaldi L, et al. Practices to optimise gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. *Vet Rec*. 2014;175(10):250-255. doi:10.1136/vr.102512

49. Sautier M, Chiron P. Challenges and opportunities for reducing anthelmintic use in ruminant livestock systems: Insights from a sheep farmer survey in France. *Prev Vet Med*. 2023;221. doi:https://doi.org/10.1016/j.prevetmed.2023.106078