Moxidectin resistance in gastrointestinal nematodes in sheep



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# Summary

This study focused primarily on the resistance of *Haemonchus contortus*, the most well known gastrointestinal nematode in sheep in the Netherlands, for moxidectin. The goal of this study was to determine the extent of the resistance problem in the Netherlands, focusing on sheep farms in Limburg, Noord-Brabant and Utrecht.

To determine the extent of the occurrence of resistance at least 20 farms need to participate. FECRT (Faecal Egg Count Reduction Test) was used to assess the efficiency of moxidectin in these herds.

During the first visit (T0) a mixed sample of faeces was collected and examined of the ewes and lambs. In case the mixed samples contained a EPG (Eggs per gram faeces) of ≥50, a second visit was planned. When visiting the second time individual faeces samples were taken (T1) and the sheep were wormed. About 10-14 days later a third visit was paid (T2) to take another individual faeces sample of the same sheep, in order to compare the EPG from this samples with the EPG of the samples taken at T1.

Eventually 19 herds of ewes and 4 herds of lambs participated in the study. In case the decrease between T1 and T2 was less than 95% and the bottom line of the 95% confidence interval was less than 90%, resistance was demonstrated. When only one of the two criteria was met, the farm was suspected of resistance. Two herds of ewes and two herds of lambs showed a reduction of 95%.

This study demonstrated the resistance for moxidectin in four herds. Two herds are suspected of resistance.

# Introduction

As in many other species gastrointestinal nematodes can cause severe problems in sheep. The most common gastrointestinal nematodes are *Haemonchus contortus, Nematodirus battus, Teladorsagia circumcincta* and different *Trichostrongylus* spp. Gastrointestinal nematode can cause different symptoms in sheep, for example diarrhea, weight loss, poor growth, pale mucous membranes and death when it is not caught in time1-5.

Most often anthelmintics are used to fight these gastrointestinal nematode. Another way to limit the number of nematode infections is to move the herd to another pasture on a regular base. Because of the lack of pastures sheep farmers usually choose to worm the sheep, leading to an increased chance of resistance. The occurrence of resistance increases in horses, goats and sheep. Resistance is demonstrated worldwide, for example in Australia, South-Africa and New-Zealand1. Anthelmintic resistance is a major problem in gastrointestinal nematodes in sheep and its occurrence appears to increase. As seen in other studies, the resistance for ivermectin in sheep is high 2-5.

Several factors play a part in the development of resistance. Due to an underestimation of the weight of the sheep or due to a wrong administration of the anthelmintics, sheep are often under dosed. Also the frequent use of anthelmintics, belonging to the same group, can lead to resistance1.  
   
Four groups of anthelmintics are used in sheep6:

* Benzimidazole,
* Imidazothiazole,
* Macrocyclic Lactones,
* Amino Acetonitril Derivates (AAD).

Ivermectin belongs to the group of macrocyclic lactones, also known as the third group of anthelmintics. Moxidectin also belongs to this group and until recently it was the only active substance for which no resistance was found in the Netherlands2-5,7,8. Last year, resistance for moxidectin was demonstrated on a farm in the middle of the Netherlands. Moxidectin seems to lose its effect9. To know which anthelmintics have and which have not lost their effectiveness, and to know to what extent this is the case, monitoring is very important. Over the years, this monitoring is carried out by different veterinary medicine students. The present study focuses on resistance to moxidectin in gastrointestinal nematodes in sheep, because recently the first case of moxidectin resistance is found9. It is not yet known whether this was an isolated incident, or that more farms suffer from moxidectin-resistant gastrointestinal nematodes in sheep. Therefore, it is important to study this subject.

The aim of this research project is to establish the percentage of sheep farms where moxidectin resistance in gastrointestinal nematodes of sheep occurs. Is the resistance for moxidectin limited to the one sheep farm found in the other study, or does it occur in more sheep farms?

More specifically, this study focused on resistance to moxidectin in sheep farms in Limburg, Noord-Brabant and Utrecht. The research focuses, on the most important gastrointestinal nematode species*, Haemonchus contortus,* in the Netherlands.

# Methods

This chapter will discuss the design, the process of data collection, the population and the measurement instruments used. In the last paragraph the analyses are described.

2.1 Design and procedure  
  
This study is a quantitative research, using monitoring to gather data to study the development of resistance for anthelmintics.   
  
Initially only acquainted sheep farmers were approached to participate in this study. With their help addresses were obtained from other sheep farmers that could be asked. Soon also the Schapenadviesgroep Provinos in Venlo was asked for cooperation, and they placed a call in the Newsletter of the LLTB (Limburgse Land- en Tuinbouwbond10). Furthermore two veterinary clinics were approached. After explaining the goal of this study, they were asked whether they knew sheep farmers that would be willing to participate. However most sheep farms were found thanks to a list of addresses of the studbook of Texelaars.

When sheep farms decided to participate, 1 to 3 visits were paid to the farm. At the first visit (T0) a mixed sample of faeces of the herd of ewes and/or lambs was collected. The mixed samples were collected by picking up fresh faeces in at least 10 different places in the pasture where the herd was located. In case the mixed sample tested positive for eggs of gastrointestinal nematode a second (T1) and third (T2) visit was paid to the farm. During the first visit the sheep farmer was, once again, informed about the purpose of the study and its procedure.

At the second visit (T1) samples of faeces were collected of 15-20 sheep and then those sheep were wormed with moxidectin (Cydectin 0,1%). The weight of the heaviest sheep was estimated in order to determine the amount of moxidectin that should be administered to all sheep. When logistic possible, a sheep weighing scale was used to determine the weight of the sheep very accurately.

The moxidectin was administered orally using a drench gun. A dose of 1 ml per 5 kg was used. All sampled animals were individually labeled and tested for the secretion of worm eggs (EPG = eggs per gram of faeces). At the first farms fresh faeces was picked up per sheep. Since this was a time consuming method, it was decided to take the faeces rectally at the rest of the farms. So eventually in most animals the faeces was taken rectally, unless the animals dropped faeces themselves. The identification numbers of the sheep were noted or scanned and the animals were marked in order to be able to find them easily later on.

Then 10-14 days later (T2) the farm was revisited to take another sample of faeces of the same sheep as T1 to determine its EPG. Through the list of identificational numbers of the sheep and through the fact that the sheep were marked, they were easy to find in the herd.

2.2 Population

The population used in this study consisted of sheep farms in Limburg, Noord-Brabant and Utrecht, which had at least 15 sheep and/or lambs. The sheep were not allowed to have been treated with anthelmintics recently (less than three weeks ago).   
In *Haemonchus contortus* the ewes secrete eggs. In the spring these eggs are spread in the pasture through the faeces of the ewes, and in May the new larvae come out. When the lambs consume these larvae it takes approximately three weeks before the eggs can be found in the faeces of the lambs6. So to enable the worm infection to develop into a patent infection, the lambs have to be in the pasture for at least three weeks.

2.3 Measurement instruments  
  
All faeces samples were tested on worm eggs by means of the McMaster technique in the laboratory.

To determine which gastrointestinal nematode species show resistance, the faeces samples from T1 and T2 (as mixed sample) were also used to grow larvae. The species of the worms can only be identified based on the infective larvae.

The techniques used are standard methods, commonly used in the veterinary parasitology. The methods used include the McMaster technique to determine the EPG and a 10-day larval culture. In the McMaster method a saturated saline solution is used. The technique has a detection limit of 50 EPG. By comparing the EPG results on T1 and T2, an egg reduction percentage can be calculated (FECR: Faecal Egg Count Reduction).

A questionnaire was used to gain further insight in the management of the sheep farms. The farmers had to answer questions about the number of animals they have, the amount of land they have, the last time they wormed the herd, the number of times they move the herd to another pasture, whether they keep sheep in quarantine before introducing them into the herd, and whether they have had problems in the past due to worms. See appendix 1.

2.4 Analysis

First the average EPG per farm was calculated, followed by a calculation of the decrease between the two average EPG’s (T1-T2).

A herd shows resistance for moxidectin when the decrease in EPG after worming is less than 95% and the bottom line of the 95% confidence interval is less than 90%. If only one of these two criteria is met, the farm is suspect for resistance11- 13.   
With Excel the confidence interval was calculated. First all reduction percentages were calculated per sheep, than the confidence interval was calculated by means of a data analysis. Based on the calculations the lower limit of the confidence interval was calculated.

# Results

3.1 Population

During this study more than 30 farms were asked to participate. Several farmers were not interested or could not participate due to various reasons, such as no time, the sheep were recently wormed, or the herds were too big. The sheep farmers that agreed to participate in the beginning, have all been helpful till the end of the study. Eventually 24 farms were visited in Limburg, Noord-Brabant and Utrecht. The types of farms varied. There were farms that had herds for hobby, for management of landscapes, to produce meat, or to produce breeding ewes. Large farms of over 550 sheep were visited as well as small farms with 15 sheep. A few farms had several herds, at those farms only one herd was sampled and wormed. If there was a herd of ewes and a herd of lambs at one farm, both herds were accepted in the study as separated herds. This leads to a participation of 24 herds of ewes and 11 herds of lambs. Of those herds 19 herds of ewes and 4 herds of lambs had an EPG of ≥ 50. For these herds the FECRT (Faecal Egg Count Reduction Test) was performed.

From April till June 18 sheep farms were visited in Limburg, 5 in Noord-Brabant and 1 in Utrecht, each with at least 15 ewes and/or lambs. The farms approached are from the circle of acquaintances, the circle of clients of some veterinary practices and from local organisations of sheep farmers.

Of some sheep farms no data are available of the third visit (T2). This is often caused by the fact that the ewes did not have any faeces that could be taken, or the ewes were already dead/slaughtered. In the lambs the causes were that they could not be caught, did not have any faeces that could be taken, or that they jumped over the fence when captured. Of these sheep the results on T1 were not included in the calculation of the average EPG.

3.2 Questionnaire  
  
The questionnaire was sent to 21 sheep farmers, 19 of them have completed it and send it back. This is a response rate of 90,48%. The questionnaire revealed that all farms were closed farms. In most farms the sheep live in the pasture all year round, except when they lamb, the farms that have meat lambs keep the lambs inside. The time at which the lambs go to the pasture differs per farm, some of them go out in March, others in May.

Furthermore, the questionnaire revealed that most farmers move the herd to a pasture where no sheep have been for quite some time, most of the time no difference was seen between the ewes with and ewes without lambs. In case there was a difference, it appeared that the lambs were moved more often than the ewes without lambs. They are moved to pastures where the ewes have not been yet. The amount of land of the farmers varied between 1 and 30 hectares.

To determine the needed dose of the anthelmintic different methods were used. Most farmers weigh or estimate the weight of the heaviest sheep, others determine the dose for every animal individually. Most farmers worm their sheep after lambing, for the breeding season or later on based on the examination of faeces samples. Often the lambs are not wormed, except when there is a specific reason or if the lambs are moved to a pasture where they have been before.

Almost none of the farmers have had previous gastrointestinal nematode related diseases in their herds.

3.3 Results McMaster  
  
First the average EPG was calculated per farm, then the decrease of the average EPG’s (T1-T2) was calculated. The table below shows the average EPG’s per sheep at T1 and T2. FECR=(T1-T2)/T1\*100%. Sheep farm 6, 15 and 22 had a negative faeces sample at T0 and are therefore not included in the rest of the study.

**Table 1: FECRT results for the Strongylus-type eggs.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Farms*** | ***Ewe/***  ***Lamb*** | ***Number of animals*** | ***EPG Strongylus***  ***T1*** | ***EPG Strongylus T2*** | ***FECR (%)*** | ***Bottom line confidence interval*** |
| 1 | Lamb | 13 | 111,54 | 0 | 100 | 100 |
| 2 | Ewe | 10 | 375 | 5 | 98,67 | **83,69** |
| 3 | Ewe | 12 | 829,167 | 8,33 | 98,99 | 98,98 |
|  | Lamb | 15 | 63,3 | 6,67 | **89,47** | **40,49** |
| 4 | Ewe | 12 | 1112,5 | 20,83 | 98,13 | 90,52 |
|  | Lamb | 10 | 85 | 5 | **94,12** | **57,94** |
| 5 | Ewe | 14 | 164,29 | 0 | 100 | 100 |
| 6 | ----- | ------ | ------ | ------ | ------ | ------ |
| 7 | Ewe | 13 | 2457,69 | 34,62 | 98,59 | 93,62 |
| 8 | Ewe | 14 | 1007,14 | 92,86 | **90,78** | **89,89** |
| 9 | Ewe | 13 | 1430,77 | 11,54 | 99,19 | 90,89 |
| 10 | Ewe | 11 | 740,9 | 22,73 | 96,93 | **47,43** |
| 11 | Ewe | 17 | 117,65 | 0 | 100 | 100 |
| 12 | Ewe | 12 | 4579,17 | 4,17 | 99,91 | 99,05 |
| 13 | Ewe | 14 | 1314,29 | 10,71 | 99,18 | 98,53 |
| 14 | Ewe | 12 | 1862,5 | 0 | 100 | 100 |
| 15 | ----- | ------ | ------ | ------ | ------ | ------ |
| 16 | Ewe | 13 | 3015,38 | 3,85 | 99,87 | 99,38 |
| 17 | Ewe | 12 | 79,17 | 0 | 100 | 100 |
| 18 | Ewe | 10 | 370 | 0 | 100 | 100 |
| 19 | Lamb | 15 | 170 | 0 | 100 | 100 |
| 20 | Ewe | 17 | 229,41 | 0 | 100 | 100 |
| 21 | Ewe | 10 | 895 | 5 | 99,44 | 98,11 |
| 22 | ----- | ------ | ------ | ------ | ------ | ------ |
| 23 | Ewe | 14 | 114,29 | 0 | 100 | 100 |
| 24 | Ewe | 17 | 26,47 | 8,82 | **66,67** | **50,76** |

Figure 1: FECR of the ewes;

Figure 1 shows that moxidectin has not lost its effect. In 22 herds of ewes the FECR is between 95 and 100%. In 1 farm the FECR is between 90 and 95%, in this farm only one ewe had a lot of eggs in her faeces after worming, this had a big impact on the decrease of the reduction percentage. In one farm the FECR is <90%, due to a very low ECG at T1. Some animals did not have any eggs in their faeces at T1.

Figure 2: FECR of the lambs;

For the herds of lambs the FECR is <90% in one farm, at this farm the lambs had only one or a few eggs in their faeces. One farm had a FECR between 90 and 95% and the other farms had 100% reduction.

Confidence interval:

The 95% confidence interval was <90 on six farms, 4 of them were herds of ewes and 2 of them were herds of lambs. This only includes farms in Limburg, the farms in Noord-Brabant had a confidence interval of >90.

# Discussion

4.1 Methodological discussion

The research was conducted in the months April, May and June. Many lambs could not be included in the study because they had not been in the pasture yet, or not long enough. However, in the faeces of the lambs that had been in the pasture for more than three weeks, often no eggs were found. The weather could have had an important influence on the lack of eggs, since it had not been very warm yet. Later on in the study several herds could not participate because they had been wormed recently.

Of all farms larval cultures were used to determine which worm species is resistant for moxidectin. Because of an initial lack of evidence for resistance in this study the cultures where not further studied to determine the species. Possibly another researcher could use the cultures to determine the species.

The results of the mixed samples often differed from the results of the individual samples.

In some farms a low EPG was found in the mixed samples whereas all animals had a high EPG in their individual samples. This could be explained by the time between T0 and T1.

The other way around was also found: a high EPG in the mixed sample and low EPG’s in the individual samples. A possible explanation could be that the mixed sample contained faeces of animals with many eggs, and those animals were not included in the group were individual samples were taken.

The fact that in some farms the sheep were weighted whereas in other farms the weight was estimated could lead to a difference in results. However, in this study no difference could be found, showing that the sheep farmers can give a good estimation of the weight of their sheep.

Based on the calculation of the confidence interval, evidence was found to conclude that in 4 herds resistance has occurred and that two herds are suspected of resistance. In two herds of lambs were the confidence interval was <90, the EPG at T1 was very low and in both farms for 1 lamb the EPG had not decreased at T2. Stating that the whole farm is resistant might be a premature conclusion. However, the administering of the anthelmintic went well in these farms and the lambs were weighted on the scale, so they have had the right dose of anthelmintic.

In the two herds of ewes, where the confidence interval was <90, a low decrease in EPG was found in a few ewes. In one farm the EPG of one ewe was decreased by only 50% and in the other farm there were two ewes that showed only a minor decrease in EPG. Both farms were visited early on in the study, so due to a lack of experience administering the anthelmintic might not have gone as smooth as it should have, may be the sheep spewed some of it. Furthermore the ewes were not weighted on a scale, but the weight was estimated.

Beside the 4 herds were resistance was demonstrated, two other herds were suspected of resistance. One herd had a low EPG at T1 and the EPG of one ewe did not decrease at all at T2. In the other herd one ewe showed an increase in EPG between T1 and T2. Again, to state that the whole herd is resistant might be a premature conclusion .

4.2 Recommendations  
  
The results of this study apply to Limburg and Noord-Brabant, these results do not imply that there is no resistance for moxidectin at all in the Netherlands. In order to determine whether there is resistance to moxidectin further research in other area’s in necessary.

In order to give the larvae more time to develop, it might be better to conduct the research later on in the year. That might also lead to more herds of lambs that can be included, because they have been in the pasture long enough to participate. A disadvantage is that the chance that the farmer already has wormed the herds increases. Therefore it would be wise to ask the farmers to participate early on, so they can wait with worming their herd.

It is best to perform this kind of research with several students instead of doing it alone. So the practical execution could be carried out faster and more farms could be approached. Examining the faeces in the lab was rather time consuming, this precious time could not be used to visit more farms.

Another point of attention is administering the anthelmintic. It is recommended that some instruction is given about estimating the weight of the animals and about administering the anthelmintic, before the first visit to a farm. When visiting the first farms, it has occurred that the anthelmintic flows out of the mouth of the sheep. Noticing that, those sheep were given some extra anthelmintics. This could have been prevented by a proper instruction beforehand.

To prevent resistance, it is better to make the farmers aware of the consequences of inaccurate use of anthelmintics. It is wise to inform them about worming based on the results of examination of the faeces of their herd.

# Conclusion

This study demonstrated the resistance of gastrointestinal nematodes for moxidectin on four sheep farms. Two other sheep farms are suspected of resistance. However, it is not possible to determine whether resistance occurred on these two farms indeed or that the anthelmintic was not administered properly. All farms that were suspected of resistance and the farms where resistance was demonstrated are located in Limburg.

For the time being it can be concluded that moxidectin still works properly in Noord-Brabant.

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# Appendix 1

Questionnaire  
  
The following questionnaire was sent to the sheep farms that participated in this study.

**Bedrijf: …**

**Vragen aan schapenhouder m.b.t. onderzoek naar anthelminticumresistentie:**

1. Wat voor type bedrijf heeft u? ❑ Vleeslam producent

❑ Fokooi producent

❑ Hobby

❑ Landschapsbeheer

1. Hoeveel dieren heeft u?

Ooien: ........

Lammeren: .......

Dekrammen: ......

1. Hoe lang houdt u ze aan?

Slachtlammeren: ...................................................................................

Ooilammeren: ......................................................................................

Anders: ...............................................................................................

1. Hoe oud zijn de lammeren op dit moment?

Variërend van .............. tot .............

1. Is het bedrijf open/gesloten? (beweiding wel/niet gezamenlijk met andere bedrijven)

..........................................................................................................................................

1. Dekram(men): eigen / aankoop / huur (gezamenlijk gebruik met andere bedrijven)

..........................................................................................................................................

1. Worden deze dekrammen gecontroleerd op EPG voordat ze op het bedrijf komen?   
   Ja / nee / weet niet

Indien nee, worden de dekrammen dan standaard ontwormd bij aankomst op uw bedrijf?

Ja / nee

Zo ja, waarmee?  
 …………………………………………………………………………………………..

1. Blijven dekrammen in quarantaine voor twee weken of niet?

..........................................................................................................................................

1. Handelt u veel of koopt u veel dieren aan? Ja/nee

Zo ja, ontwormt u deze dieren dan en waarmee?

..........................................................................................................................................

1. Blijven de schapen het hele jaar buiten (uitgezonderd de lammerperiode)  
   Ja / nee
2. Wanneer zijn de lammeren op het land gekomen?

.....................................................................................

……………………………………………………………

1. Hoeveel land heeft u tot uw beschikking waarop de dieren weiden?

....... ha/bunder

1. Verweidt u de dieren regelmatig?

Ja/nee

Zo ja, hoe?

.............................................................................................................................................

....................................................................................................................

1. Is er een verschil van verweiden tussen ooien met lammeren en ooien zonder lammeren?  
   Ja / nee  
   Zo ja, wat is het verschil?  
   ....................................................................................................................................
2. Wanneer heeft u de dieren voor het laatst ontwormd?

..........................................................................................................................................

1. Waarmee heeft u ze ontwormd? (zie tabel 1)

Ooien: ..............................................................................................................................

Lammeren: ......................................................................................................................

1. Hoe bepaalt u de dosering van het wormmiddel:  
   Wegen / schatten op basis van individuele dier / op basis van gemiddeld gewicht / op basis van het zwaarste dier / naders, namelijk:  
   ………………………………………………………………………………………
2. Op welke tijdstippen ontwormt u de dieren standaard:

- De ooien: nooit / bij aflammeren / bij naar buiten gaan / bepaald interval / bij spenen /

voor het dekseizoen / bij opstallen / op geleide van mestonderzoek / anders, namelijk:

............................................................................................................

- De lammeren: nooit / bij spenen / vaste intervallen / op geleide van mestonderzoek /

anders, namelijk:

............................................................................................................

1. Is de behandeling voor zover u kan nagaan effectief? Ja / nee / anders, namelijk:

..........................................................................................................................................

1. Wordt dat gecontroleerd door mestonderzoek? Ja / nee

Zo ja, door een dierenarts of een laboratorium?

..........................................................................................................................................

1. Heeft u in de koppel ondanks ontwormen last van maagdarmwormziekte gehad?

Ja / nee

Zo ja welke klachten en wanneer, indien bekend, welke wormen?

.................................................................................................

Is dit bevestigd door een dierenarts?

Ja / nee