# Moxidectin resistance of *Haemonchus contortus* in Dutch sheep

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## Abstract

*Haemonchus contortus, also known as the red worm of the abomasum, is a gastrointestinal parasite of sheep. This pathogenic nematode is of great importance in the sheep sector because of the severe clinical signs and upcoming resistance of H.contortus to moxidectin in the Netherlands. The first case of moxidectin resistance to H.contortus in the Netherlands was reported in 2012 on a farm in the province of Zuid-Holland. In 2013 moxidectin resistance to H.contortus was found in the Netherlands in the province of Limburg. This study attempted to find out whether there is moxidectin resistance to Haemonchus contortus in sheep in other provinces in the Netherlands. It is of great importance to find out if this is the case, because of the widespread resistance of other anti-parasitic drugs like ivermectin and doramectin. If moxidectin resistance is proven, then there will be a lack of other effective anti-parasitic drugs for sheep. The focus in this study was on three provinces in the Netherlands; Utrecht, Overijssel and Gelderland respectively. The Fecal Egg Count Reduction Test (FECRT) was used to determine the number of Strongylus type eggs ten to fourteen days after the administration of moxidectin. Only one farm in the province of Gelderland has moxidectin resistance to H.contortus. Another farm in the province of Utrecht is suspected of resistance.*

## Introduction

*Haemonchus contortus*, also known as the red worm of the abomasum, is a gastrointestinal parasite of sheep. This pathogenic nematode is of great importance in the sheep sector because of the severe clinical signs and upcoming resistance of *H.contortus* to moxidectin in the Netherlands[3,6]. The first case of moxidectin resistance to *H.contortus* in the Netherlands was reported in 2012 on a farm in the province of Zuid-Holland[3]. In 2013 moxidectin resistance to *H.contortus* was found in the Netherlands in the province of Limburg[6].

*H.contortus* worms are the main cause of parasitic related death in sheep. This is the reason why *H.contortus* is considered to be the most important parasite in sheep[4]. Animals infected with a lot of *H.contortus* larvae develop severe anemia due to the fact that the parasites feed on blood from the abomasal mucosa of the host[4]. Each adult worm is able to feed on 0.05 millilitre blood each day[9]. Sheep with anemia have pale mucous membranes of the eyes and mouth. Anemia often leads to a lack of cobalt[4,9]. Oedema of the lower jaw, poor lamb growth and hard dry faeces can also be seen[4]. Sometimes infections with this parasite lead to per acute death because of haemorrhagic gastritis[4,9]. Opposed to other gastrointestinal parasites, *H.contortus* infections do not lead to clinical signs such as diarrhea[4].

*H.contortus* life cycle takes about twenty-one days. *H.contortus* worms can lay around 5000 eggs each day[4,9]. A sheep with 5000 *H.contortus* may lose about 250 ml blood daily[9]. Infected animals will shed eggs via the faeces. Once on the land, the larvae in the eggs develop into $L_1$, $L_2$ and hatch. They then develop into $L_3$ and migrate to the tips of the grass to be easily taken up by the grazing animals. This process takes about two weeks in the Netherlands. Optimal conditions for the $L_1$ larvae to hatch and migrate to the soil are several rainy days with a warm temperature, like those that usually occur in the early spring[4]. From the moment the larvae hatch and develop, grazing animals will eat the $L_3$ and become infected. The $L_3$ larvae on the soil will be infectious till Autumn[4]. Once taken up by the animals, the larvae end up in the abomasum, then molt into $L_4$ and eventually become adults that are able to lay eggs. The other possibility is that the larvae go in hypobiosis when conditions are not conducive for the entire life cycle to be completed. A favourable condition for further development of $L_3$ larvae is an increased estrogen level of the host for example[4]. The greatest risk of lambs to become infected is in the summer and spring, when there are plenty of $L_3$ larvae on the grass waiting to get eaten. *H.contortus* larvae are able to survive the winter in the abomasum of sheep. They hibernate and become active two to four weeks prior lambing and start laying eggs in the pregnant ewes[4,9].

This study attempted to find out whether there is moxidectin resistance to *Haemonchus contortus* in sheep in other provinces than Limburg and Zuid-Holland in the Netherlands. It is of great importance to find out if this is the case, because of the widespread resistance of other anti-parasitic drugs like ivermectin and doramectin[5,10]. If moxidectin resistance is proven, then there will be a lack of other effective anti-parasitic drugs for sheep. There is some degree of cross-resistance between moxidectin and avermectins, although the resistance of these drugs is not identical[5]. In 2001 moxidectin resistance to *T.circumcintca* was proven for the first time in Scotland[8]. Moxidectin resistance to *H.contortus* has also been found in Germany[7]. In other words, moxidectin resistance is developing in Europe.
The focus in this study was on three provinces in the Netherlands; Utrecht, Overijssel and Gelderland respectively. The Fecal Egg Count Reduction Test (FECRT) was used to determine the number of *Strongylus* type eggs ten to fourteen days after the administration of moxidectin.

## Materials and Methods

*Farms*

To get in touch with sheep farmers, veterinary practices in Meteren and Nijkerk (both in the province of Gelderland) were contacted by phone at first. Addresses of the sheep farmers that would like to participate in this study were given by the veterinarians. Some farmers in Hengelo (Overijssel) and Werkhoven (Utrecht) are acquaintances and were approached to participate in this study, during a visit. A few conditions were made for farms to participate: the farmer should have at least fifteen lambs and he or she should not have treated the animals with an anti-parasitic drug for the last weeks, depending on the drug (if moxidectin was used, the animals should be minimally treated eight weeks ago). Finally, eleven farmers participated in this study.

*Appointments and selecting farms*

The first appointment ($T_0$) was about taking a fresh mixed faeces sample off the ground from lambs (with a minimum of ten individual faeces samples). These faeces samples were mixed to form a homogenous mass. Only lambs born in 2013 were used in this study. All farms were visited and mixed faeces samples were taken. The samples were examined by the McMaster technique and an EPG was determined of *Strongylus* type eggs (these *Strongylus* type eggs may contain for example *H.contortus*, *T.circumcincta* etcetera; see figure 1). If *Strongylus* type eggs were found in the sample (a minimum EPG of fifty eggs), a second appointment ($T_1$) was made with the farmer as soon as possible.
During the second appointment ($T_1$) fifteen lambs were selected from the same flock from which the mixed faeces sample was taken. No difference was made between ram and ewe lambs. The ear numbers were written down from each individual lamb and the lambs were treated orally with Cydectin© 0.1%. The heaviest lamb was chosen and its weight was estimated. This weight was used for all the lighter animals to be sure that no animals were underdosed. Per 5 kilogram body weight 1.0 millilitre Cydectin© 0.1% was used. At the same time the animals were orally treated with Cydectin© 0.1%, faeces were collected from the rectum by one finger. Fifteen faeces samples were collected and examined by the McMaster technique in the laboratory.
Ten to fourteen days later the third appointment ($T_2$) was made. During this last appointment faeces were collected from the same fifteen lambs. These faeces samples were individually examined by the McMaster technique. Finally, the Fecal Egg Count Reduction Test (FECRT) was carried out to determine the possibility of moxidectin resistance.

*McMaster technique*
Three gram faeces, forty-two millilitre saturated salt solution (1.18-1.20 g/ml), a counting chamber, a mortar, a pounder, falcon tubes (50ml), a strainer, a spatula, a bowl, a scale and a Pasteur pipette are needed. Three gram faeces is weighed and mixed in a falcon tube with a few millilitres salt solution. Next the mixture is put in the mortar and is triturated with the pounder. The remaining salt solution is added and the mixture mixed. Then the mixture is poured over the strainer and ends up in a bowl underneath the strainer. With a Pasteur pipette some of the mixture is collected and added to the counting chambers. Allow the chamber to stand for a few minutes so the eggs have to time to float. Then the chamber is viewed under the compound microscope at 10 x 10 magnification. *Strongylus* eggs are counted in the chambers. The eggs outside the chamber should be ignored. The number of eggs found in both chambers has to be added and multiplied by fifty. The result is the number of eggs per gram faeces (EPG).

*Fecal Egg Count Reduction Test and the lower limit of the confidence interval*
To determine the reduction of *Strongylus* type eggs after oral moxidectin administration during the third appointment ($T_2$) the FECRT was carried out according to the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP)[2)]. The following formula was needed:

$FECRT = (T_1-T_2)/T_1 * 100\ \%$

$T_1$: *EPG from each animal not treated with Cydectin© during the second appointment*
$T_2$: *EPG from the same animal treated with Cydectin© ten to fourteen days later during the third appointment*

Two rules are important to determine if an assumption of resistance can be made:

1: ''The mean FECR% is < 95%''
2: ''The lower limit of the confidence interval is < 90%''

If only one rule is fulfilled then there is a suspicion of resistance.
If two rules are fulfilled then there is resistance.

*Breeding of the larvae*
If an EPG of *Strongylus* type eggs is found after treatment with Cydectin© then the larvae species should be identified. *Strongylus* type egg positive faeces of all the lambs from the farm were mixed and put in an open jar so oxygen could reach the eggs. After nine days lukewarm water was added and the jar was put upside down on a petri dish. One day later the amount of water in the petri dish was transferred into a falcon tube. The petri dish was rinsed and the rinse water was also put in the falcon tube with the rest of the solution. The falcon tube was fully filled with water. After two hours the larvae have sunk and the water has to be removed while a few millilitres of water remained in the falcon tube. Then again the same falcon tube was filled with water. Two hours later the water was removed until a few millilitres remained. This solution was pipetted and transferred on several glass slides and examined under the microscope to identify the species. One hundred larvae were examined under the microscope. Figure 2 shows a *H.contortus* larve.

***Figure 1:*** *Eggs found under the microscope with the Centrifugation Sedimentation Flotation technique: 1. Trichuris ovis; 2. Strongylus type egg; 3. Nematodirus battus; 4. Strongyloïdes papillosus. (Faculty of Veterinary Medicine, Utrecht, the Netherlands).*

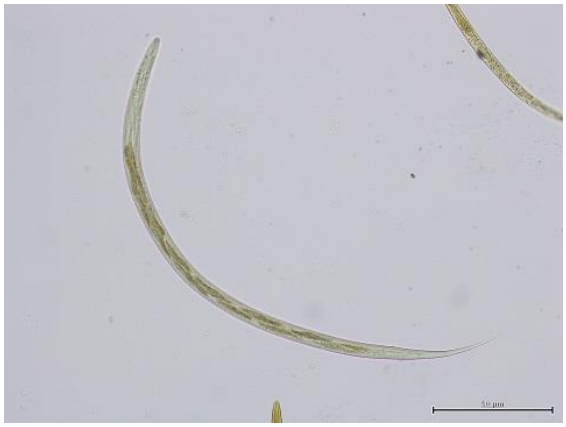

***Figure 1:*** *Haemonchus contortus larva after breeding of the Strongylus type eggs from farm one. Note the sixteen intestinal cells. This is typical to determine the species. (Faculty of Veterinary Medicine, Utrecht, the Netherlands, 2013).*

*Questionnaire*

Each participating farmer received a questionnaire to get an impression of the farm. Questions about the amount of land, amount of animals, type of farm etcetera were given. This can give some clues about the possibility of resistance of the anti-parasitic drug. If the farmer treats the animals very often with moxidectin then the possibility of resistant parasites can be high for example[10]. The questionnaire can be found in the annex.

## Results

*First appointment $T_0$*

In total eleven farms were visited. Eight of the eleven farms had a *Strongylus* positive mixed faeces sample. These eight farms were involved in this study to determine if there is a chance of moxidectin resistance to *H.contortus* (see table 1).

***Table 1****: Farms at the first appointment ($T_0$)*

| Farms | Province | *Strongylus* positive/negative mixed faeces sample | *EPG from the mixed faeces sample* |
|---|---|---|---|
| 1.As | Gelderland | Positive | 1000 |
| 2.Do | Gelderland | Positive | 100 |
| 3.Ve | Gelderland | Positive | 50 |
| 4.Pi | Gelderland | Positive | 700 |
| 5.Dij | Utrecht | Positive | 150 |
| 6.Bo | Utrecht | Positive | 300 |
| 7.Ec | Utrecht | Positive | 1750 |
| 8.Go | Overijssel | Positive | 150 |
| 9.Bl | Gelderland | Negative | 0 |
| 10.Mo | Gelderland | Negative | 0 |
| 11.Ho | Utrecht | Negative | 0 |

*Second appointment $T_1$ and third appointment $T_2$*

During the second appointment fifteen animals were treated with Cydectin© 0.1% and faeces was collected rectally. All farms were *Strongylus* positive and an EPG from each individual animal was calculated. Ten to fourteen days later the faeces were collected rectally from the same lambs and again an EPG from each individual animal was calculated. See the results in table 2.

The number of lambs that participated during the third appointment was often different than during the second appointment. While catching the animals, the animals would defecate because of stress. That is why often the rectums were empty and no data was available. In table 2 the number of animals per farm is listed that participated in the second and in the third appointment (faeces was collected on both times).

*FECRT (on $T_2$) and lower limit of the confidence interval*

In table 2 the FECR% and the lower limit of the confidence interval are listed.

The FECR% of the second farm is zero. The lower limit of the confidence interval could not be calculated because of the greater EPG on $T_2$ (during the third appointment, ten to fourteen days after the administration of Cydectin©) than the EPG on $T_1$ (during the second appointment, before treatment with Cydectin©). The larvae have been determined from farm two. One hundred percent of the larvae were *H.contortus.* On this farm moxidectin resistance of *Haemonchus contortus* is proven.

The lower limit of the confidence interval of farm seven is < 90%. This farm is suspected of moxidectin resistance (see table 2), but it is unknown to which parasite there could be resistance. *Strongylus* type eggs were found on $T_2$, but no larval breeding was performed to determine the parasite species because there was not enough faeces left.

Farm eight is not suspected of resistance. Although an EPG of five was found on $T_2$, the mean FECR% is > 95 % and the lower limit of the confidence interval is > 90 %. Neither one

of the rules is fulfilled to prove resistance statistically.

***Table 2**: All participating farms and the results*

| Farm | Animals | Mean EPG $T_1$ | Mean EPG $T_2$ | Mean FECR% | Lower limit of the Confidence Interval % |
|---|---|---|---|---|---|
| 1.As | 11 | 582 | 0 | 100 | 100 |
| 2.Do | 15 | 439 | 973 | 0 | -- |
| 3.Ve | 13 | 24 | 0 | 100 | 100 |
| 4.Pi | 15 | 257 | 0 | 100 | 100 |
| 5.Dij | 12 | 247 | 0 | 100 | 100 |
| 6.Bo | 13 | 644 | 0 | 100 | 100 |
| 7.Ec | 12 | 469 | 13 | 97.3 | 84.0 |
| 8.G | 12 | 321 | 5 | 98.7 | 97.0 |

## Discussion

First, the results will be discussed, followed by the practical difficulties encountered during this study and other issues.

One farm has moxidectin resistance to *H.contortus* and one farm is suspected of resistance.
There is no discussion about the second farm in the province of Gelderland with the resistance. After treatment there were more *Strongylus* type eggs found in the faeces of the lambs than before treatment. After culturing the larvae, 100 % of the larvae were identified as *H.contortus*. Also, the farmer already suspected an ineffectiveness of moxidectin and therefore gladly participated in this study. The farmer used Cydectin© several years and did not use a grazing schedule (changing pasture every two weeks) to prevent the contamination of sheep with parasite eggs (written in the questionnaire). The animals often ended up on parasite contaminated land. The high chance of re-infection on contaminated land and the frequent use of anti-parasitic drugs, like Cydectin©, are risk factors for developing resistance[10)].

The seventh farm is suspected of resistance. The lower confidence interval is 84 percent. This is less than 90 percent. Only one of the twelve animals from this farm had an EPG of 150 after treatment. All the other animals had an EPG of zero. The same animal had an EPG of 250 before treatment. The chance that only this animal did not receive enough moxidectin or he/she spit it out is considered to be high. Also the farmer uses a grazing schedule (every two weeks change of pasture) to prevent contamination of sheep with parasites and the animals are only treated with an anti-parasitic drug (not Cydectin©) based on the results of faeces examination. The chance of resistance is considered small.

This study took place in the late spring and summer. In this period most of the lambs were weaned and automatically treated with an anti-parasitic drug. These farmers could not participate because they did not meet one of the conditions (animals should not be treated for the last few weeks depending on the anti-parasitic drug). The other condition was that there should be at least fifteen lambs. A lot of farmers sell their lambs resulting in non-steady groups. There were also farmers that would not like to participate because of the workload (catching the animals for example).

Catching the sheep causes a lot of stress. The lambs begin to defecate and often their rectum is empty or less than three gram faeces is received from the animal. Getting faeces of the ground is risky because of the high chance of getting the wrong faeces sample. Catching the animals a few hours prior to treatment with Cydectin© could reduce the stress. They get used to their surrounding and even if they defecate on the moment of catching, they produce some new faeces later on. The disadvantage is that the workload of the farmer increases and depending on the circumstances, the animals cannot eat or drink for a few hours.

Another problem concerns the ear tags. Treating the animals and getting faeces samples are stressful moments. It is difficult to take note of the ear numbers because the lambs are trying to get away by jumping and kicking. The ear numbers are small and sometimes the ear tags are lacking. When the lambs did hide or got away there was no time to see the number. It is better to mark the animals with numbers on their back. For example, Raidex© colour spray is very effective and is clearly visible on the backs of the lambs. Big numbers can be drawn and easily recognized, even if the lambs are running and

jumping. Heavy or prolonged rainfall can wash the marks off. This could be a problem.

The weight of the biggest lamb was estimated on each farm and used for each lamb to determine the dosage of the anti-parasitic drug. It would be better to weigh the lambs to be more accurate to prevent under dosing of the anti-parasitic drug. This is also important to prevent resistance of anti-parasitic drugs[10)].

One out of the eight farmers (12.5%) had sheep with resistance of moxidectin to *H.contortus* in this study. In 2013 there were also four farms found with resistance of moxidectin to *H.contortus* in the province of Limburg. This is comparable to other countries like Germany[7)]. Also in other countries, for example Scotland, moxidectin resistance is developing[8)]. Even if there are only a few farms with resistance of moxidectin in the Netherlands, one should not forget that resistance of moxidectin is still developing. It is important to consider this as a serious problem because of the ineffectiveness of other anti-parasitic drugs[5,10)].

Treating sheep with anti-parasitic drugs only when faeces examination is performed and preventing the animals from eating parasite contaminated grass are good measures to prevent or slow down the developing resistance. Weighing the animals to prevent under dosing and not always treating them with the same anti-parasitic drug are also great actions. When selling the animals with moxidectin resistance to *H.contortus*, there is a possibility that these animals will shed eggs with the resistant parasites and other flocks can get infected. This should be noted when selling the animals not meant for slaughter.

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## Annex

**Questionnaire**

**Contactinformatie**
Contactgegevens bedrijf

Bedrijfsnaam:

Contactpersoon:

Adres:

Postcode:

Telefoonnummer:

E-mailadres:

**Contactgegevens onderzoekers**
-
-

**Vragenlijst m.b.t. onderzoek naar anthelminticumresistentie**
*Omcirkel het juiste antwoord, meerdere antwoorden mogelijk*

Soort bedrijf: vleeslamproducent / fokkerijbedrijf / zuiglamproducent / jaarrondproducent / weidelambedrijf / (natuur)begrazingsbedrijf / hobbybedrijf (<31 dieren)/ anders:

Aantal dieren op het bedrijf:

Aantal ooien:

Aantal lammeren:

Hoe lang houdt u uw dieren gemiddeld aan;
Slachtlammeren:

Ooilammeren:

Hoe oud zijn de lammeren;
Variërend van tot

Speendatum:

Wat is de *exacte* datum dat de lammeren op het land gekomen zijn:

Hoeveel (hectare) land gebruikt u om de dieren te weiden:

In hoeveel percelen is het opgedeeld:

Wordt er gebruik gemaakt van een omweidingsschema: JA/NEE

Hoe lang verblijven de dieren gemiddeld op één perceel:

Hoeveel tijd zit er tussen totdat de dieren weer terug komen op een eerder dit jaar gebruikt perceel:

**Bedrijfsvoering**
*Omcirkel het juiste antwoord*
Is het bedrijf open of gesloten: OPEN/GESLOTEN

Maken meerdere bedrijven gebruik van dezelfde wei(des): JA/NEE

Worden er dieren op het bedrijf aangekocht: JA/NEE

Zo ja, welke:

Worden de aangekochte dieren gecontroleerd
op EPG (=wormeieren per gram mest)
voordat ze op het bedrijf komen: JA/NEE

Worden aangekochte dieren standaard ontwormd
voordat zij op het bedrijf komen: JA/NEE

Zo ja, met welk middel:

Wordt de werking van een ontwormingsmiddel gecontroleerd via mestonderzoek? JA/NEE

Hoe lang blijven aangekochte dieren in quarantaine:

Wanneer heeft u de dieren voor het laatst ontwormd (*exacte datum graag vermelden):*
*Omcirkel het juiste antwoord, er zijn meerdere antwoorden mogelijk*

**Ooien**: Nooit / bij aflammeren / bij naar buiten gaan / vaste intervallen /op geleide van mestonderzoek / anders namelijk

Datum/Data van ontwormen:

**Lammeren**: Nooit / bij aflammeren / bij naar buiten gaan / vaste intervallen /op geleide van mestonderzoek / anders namelijk

Datum/Data van ontwormen:

**Dekrammen**: Nooit / bij aflammeren / bij naar buiten gaan / vaste intervallen /op geleide van mestonderzoek / anders namelijk

Datum/Data van ontwormen:

Wat doet u wanneer u denkt te merken dat uw dieren, ondanks ontwormen, toch erg last hebben van maagdarmwormen:

Heeft u uw bedrijfsvoering m.b.t. preventie maagdarmwormziekte in de loop der jaren veranderd:
Zo ja, hoe:

Overige opmerkingen bedrijfsvoering:

Opmerkingen over de enquête: