Prevalence of endoparasites in rabbits from Dutch rabbit fanciers

Master's Research Project

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Summary

The prevalence of the coccidium *Eimeria* has been extensively studied in commercially farmed rabbits and to some degree in private-owned rabbits. *Eimeria* spp. can lead to significant morbidity and mortality, especially in young rabbits. Fewer studies have determined the prevalence of *Passalurus ambiguus* in rabbits. This nematode is generally thought to be non-pathogenic. Currently, there is a lack of knowledge about endoparasite infestations in rabbits in the Dutch pet industry. Therefore, this study investigated the prevalence of endoparasites, specifically *Eimeria* spp. and *P. ambiguus*, in rabbits from Dutch rabbit fanciers. Faecal samples from 191 Dutch show rabbits were tested by CSF and McMaster analysis, and a questionnaire was sent to the rabbit fanciers to assess risk factors. *Eimeria* spp. prevalence was found to be 84.3% and *P. ambiguus* prevalence was 26.7%. This is markedly higher than expected. Prevalence of *Cestoda* and *Trichuris* spp., in contrast, was low (0.5%). The implementation of quarantine measures by the rabbit fanciers when new rabbits are acquired was significantly associated with the presence of *Eimeria* spp. and *P. ambiguus* in the faeces, reducing the likelihood of either parasite being present. This indicates the importance of these measures in practice. The OPG (oocysts per gram of faeces) of *Eimeria* spp. was significantly higher if the rabbit fancier uses a cleaning agent when cleaning the rabbits' cages. This finding requires additional research to confirm and explain the phenomenon.

1. Introduction

Rabbits can be infected by a number of different endoparasites. Among the most frequently encountered are *Eimeria* spp. and *Passalurus ambiguus*. *Strongylus spp.* and *Trichuris leporis* can be found as well (Raue et al, 2017). Infections with the protozoa *Cryptosporidium cuniculus* and *Giardia intestinalis* in rabbits have been studied because of their link to human health (Zhang et al, 2012).

1.1. Endoparasites of the pet rabbit

Coccidia, a type of intracellular protozoal organism, cause coccidiosis in rabbits and other species. Coccidia are host-specific and most often belong to the genus *Eimeria* (Lebas et al, 1997). In rabbits, ten intestinal species of *Eimeria* have been identified: *E. coecicola*, *E. exigua*, *E. flavescens*, *E. intestinalis*, *E. irresidua*, *E. magna*, *E. media*, *E. perforans*, *E. piriformis*, and *E. vejdovskyi*. In addition, *E. stiedai* infests the liver. The intestinal species each have a predilection for different parts of the intestines and different depths in the mucosa (Pakandl, 2013). Moreover, there are differences in pathogenicity between these species, which range from apathogenic (*E. coecicola* and *E. exigua*) to very pathogenic, causing diarrhoea and mortality, even in low doses (*E. intestinalis* and *E. flavescens*). Infection occurs by ingestion of sporulated oocysts that are excreted with the faeces (Lebas et al, 1997). Even clinically healthy rabbits can shed *Eimeria* oocysts (Oglesbee & Jenkins, 2012). Whether or not infection leads to clinical disease is dependent on several factors, including hygiene and stress (Lebas et al, 1997). Clinical signs, including failure to gain weight or weight loss, diarrhoea, poor food conversion, anorexia and anaemia, are most often observed in young rabbits, but older rabbits may show signs of disease as well (Jithendran, 1996; Lebas et al, 1997). Coccidia can be detected in a wet mount or faecal flotation, but results are not always easy to interpret. If an animal dies before the coccidial cycle is complete, there may be no oocysts present in the faeces. Similarly, low numbers of oocysts may be found in case of infection with highly pathogenic species of coccidia, with death occurring shortly after the onset of clinical signs. In contrast, in case of infection with mildly pathogenic species of *Eimeria*, high numbers of oocysts may be found in the faeces of seemingly healthy rabbits (Lebas et al, 1997).

P. ambiguus is an oxyurid nematode that infests the caecum and colon of the rabbit. This pinworm is thought to be non-pathogenic (Varga, 2013), although there are reports that suggest *P. ambiguus* infection might be linked to poor breeding performance and poor condition of rabbits (Düwel & Brech, 1981). *P. ambiguus* has a direct life cycle and rabbits are infected by faeco-oral transmission (Pritt et al, 2012). Eggs are infective when excreted with the faeces. By eating their caecotrophs, rabbits re-infect themselves (Boecker, 1953). Other endoparasites principally infect wild rabbits and are unlikely to be found in pet rabbits. These include *Graphidium strigosum* and *Trichostrongylus retortaeformis* (Varga, 2013). The nematode *G. strigosum* belongs to the Haemonchidae, the rabbit is its natural host (Massoni et al, 2011). *T. retortaeformis* infests the small intestine of the rabbit (Audebert et al, 2002). In Europe, the whipworm *T. leporis* is also primarily found in wild rabbits (Pritt et al, 2012).

While the prevalence of *Eimeria* spp. has been extensively studied in commercially farmed rabbits, there is a lack of data on the prevalence of coccidiosis or other endoparasitic infections in the (Dutch) pet industry. The studies that have been performed in other countries suggest great differences in prevalence between pet rabbits and commercially farmed rabbits. For example, *Eimeria* spp. prevalence was found to range from 21.2% (Raue, 2017) to 27% (Mäkitaipale et al, 2017) in family-owned pet rabbits in Germany and Finland, whereas in commercial rabbit farms in Taiwan, Indonesia, and Nigeria, it ranged from 41.7% to 78.6% (Li et al, 2010; Hamid et al, 2019; Ola-Fadunsin et al, 2019). In pet shops in Taiwan, Turkey, and the Netherlands, prevalences ranged from 46.2% to 95% (Li et al, 2010; Sürsal et al, 2014; Pupping, 2019). A study conducted among Dutch animal shelters found the prevalence of *Eimeria* spp. to be 72% in official and 89% in private shelters (Hulsinga, 2020). Fewer studies have investigated the prevalence of *P. ambiguus*, which ranged from 3% to 5.7% in Finland, Germany, Turkey, and Egypt (Mäkitaipale et al, 2017; Raue et al, 2017; Sürsal et al, 2014; Elshahawy & Elgoniemy, 2018).

1.2. Aim of the study

To the author's knowledge, no studies have been performed on the prevalence of endoparasites in the Dutch population of rabbits that are kept and bred as part of a hobby. It is estimated that there are about 3.800 breeders of pet rabbits in the Netherlands. Nearly all of these are rabbit fanciers that breed rabbits as part of a hobby (Van Heijst et al, 2015). A number of the rabbits that they breed are sold as family-owned pet animals to the public. Many of the rabbit fanciers take their animals to rabbit shows. At these shows, large numbers of animals are present, possibly exposing the rabbits to stressful conditions. Because of this, they might be at a greater risk of spreading endoparasite infestations.

Since currently there is a lack of knowledge on the prevalence of endoparasites in the Dutch pet rabbit population, a study was set up to determine the prevalence of coccidiosis and helminth infestations in various segments of the pet industry. In previous years, the prevalence of *Eimeria* spp. was studied in Dutch pet shops and in Dutch animal shelters (Pupping, 2019; Hulsinga, 2020). This study will focus on rabbit fanciers that breed rabbits as part of their hobby, and aims to 1) determine the prevalence of *Eimeria* species, *P. ambiguus* and other endoparasites in rabbits from Dutch rabbit fanciers; and 2) determine to what extent these endoparasites are associated with onset of clinical disease, including the risk factors associated with it.

Based on previous research, it is expected that the prevalence of *Eimeria* spp. averages that of commercial breeders and privately-owned rabbits, i.e. approximately 50%, whereas the prevalence of *P. ambiguus* is expected to be around 5% based on the previous studies (Raue et al, 2017; Mäkitaipale et al, 2017; Li et al, 2010; Hamid et al, 2019; Ola-Fadunsin et al, 2019; Sürsal et al, 2014; Pupping, 2019; Elshahawy & Elgoniemy, 2018). These numbers have subsequently been used in the sample size determination.

2. Materials and Methods

2.1. Ethical approval

Since experimental procedures in this study were limited to an external clinical examination of the rabbit and collection of non-invasive faecal samples (which were collected from the rabbits' cages), the study in itself did not classify as an animal experiment under the Laboratory Animal Act. Prior CCD-approval was therefore not required. However, the rabbit fanciers were required to fill out an informed consent prior to examining the rabbit and collecting their faeces.

2.2. Animals

Faeces was collected from rabbits from Dutch rabbit fanciers. Since many of these fanciers regularly attend rabbit shows, it was decided to collect samples during one of these shows. Based on a sample size calculation using OpenEpi, using a prevalence of 5 (*P. ambiguus*) to 50% (*Eimeria*), a population size of 2500, a precision of 7.5% and a significance level of 5%, it was determined that at least 160 rabbits needed to be sampled. Rabbits were physically examined at the show on January 9, 2020 to determine whether they were clinically healthy and whether clinical signs indicative of parasitosis were present. Apart from a general impression, the fur, ears, eyes, nose, perineal area and faeces of the rabbits were examined. Any clinical signs that were noted, were filled out on a form which can be found in Appendix I. A total of 8 people each examined 25 rabbits, and 1 person examined 20 rabbits.

2.3. Sample collection and storage

Sampling included the collection of faecal samples from 220 rabbits from their cages at the show. The rabbis were randomly selected prior to the show by the province of the Netherlands they were from, and one rabbit was selected per rabbit fancier. Only the normal faecal pellets were collected, since caecotrophs are usually consumed directly from the anus (thereby requiring additional measures to be taken to enable collection of caecotrophs) and because 'it is generally accepted that caecotrophs eaten from the anus do not contain infectious oocysts' (Oglesbee & Jenkins, 2012). For each rabbit, one faecal container was filled with faecal pellets. These containers were stored in a refrigerator until they were examined.

2.4. Sample analysis

Sample analysis was started 4 days after the sampling moment and took approximately 10 weeks. Protocols for the methods used, as described below, can be found in Appendix II. Since on multiple occasions we could not collect enough faecal pellets to perform a CSF and McMaster analysis with both 3 g of faeces, we decided to leave out the sample if there was too little faeces to perform both a CSF and a McMaster analysis.

2.4.1. CSF analysis

In order to determine the parasitic load in the samples, a faecal flotation was performed using the centrifugation-sedimentation-flotation (CSF) technique. We decided to use 1.5 g of faeces at a minimum for the CSF analysis and to use the rest of the sample for the McMaster method. Slides were examined at 400x magnification. Each slide was systematically examined in its entirety and parasite species found were recorded. Since *Giardia* spp. are visible at 1000x magnification, it was decided not to look for these protozoa.

2.4.2. McMaster analysis

In addition to the qualitative assessment (which species are present) as performed by the CSF analysis, the McMaster method was used to obtain quantitative data on parasitic load. For the McMaster analysis, we decided to use at least 0.3 g of faeces. The McMaster counting chamber was examined systematically at 100x magnification. The number of eggs and oocysts found for each parasite species was recorded. Because time was limited, we decided to count only one counting chamber of the McMaster slide for each sample and to only perform a McMaster analysis if the CSF analysis was positive. For the same reason, it was not determined which *Eimeria* species were present.

The EPG was determined by McMaster analysis for samples positive for *P. ambiguus*. Eggs of this pinworm are shed in a circadian rhythm, the highest number of eggs being excreted in the afternoon and night hours (Rinaldi et al, 2007). This means that the EPG cannot be connected directly to worm burden. Therefore, the EPG was not included in the statistical analysis.

2.5. Risk factor analysis

Additionally, a questionnaire (Appendix III), which included questions related to presence of clinical signs, housing, hygiene, introduction of new animals, group composition and other stress factors, was sent to the rabbit fanciers whose rabbits we had examined to enable risk factor analysis. Each rabbit fancier was sent a questionnaire form with the number we had given his or her rabbit (1-220) on it, so we could trace the response back and link it to the data of the faecal analysis of that specific rabbit. For the risk factor analysis, the responses from breeders of whom we had examined multiple animals were used for each of these rabbits. Not all questions in the questionnaire were obligatory. General information on the rabbits, such as age and gender, was also collected and included in the risk factor analysis.

2.6. Statistical analysis

For the statistical analysis, the program R (version 3.4.3; R core team (2017)) was used. Binary variables are displayed as range (median), unless stated otherwise. A significance level of $p < 0.05$ was determined. Factors with $0.05 < p < 0.10$ were considered a trend towards significance. The prevalence of *Eimeria* spp. and *P. ambiguus* was determined and confidence intervals were calculated. A multivariable logistic regression was performed to determine whether there was a correlation between *Eimeria* spp. presence and the risk factors associated with disease, and the same for *P. ambiguus*. A linear regression was performed to determine whether there was an association between the *Eimeria* OPG and the risk factors for disease. For this model, log-transformed data were used, since the OPG was not distributed normally. Variables to include in the risk factor analysis were selected in two ways. A Pearson's Chi-square test was performed for each of the predictor variables as an initial screening to determine possible association with the outcome variables. Then, AIC values were calculated for the variables with $p < 0.25$, and factors that did not contribute to the model fit, i.e. they increased the AIC value of the model, were excluded from the final models.

3. Results

3.1. Study population

As stated before, initially one rabbit was selected per rabbit fancier, and an equal number of rabbits was selected from each province of the Netherlands. Due to an administrative error, however, in some cases 2, 3 or 4 rabbits were selected that belonged to the same rabbit fancier, and the provinces were not evenly represented in the study (Table 1). For each rabbit, the number tattooed in the ears was recorded to determine the year of birth. A total of 187 rabbits (85.0%) were between 0 and 1 years old, 26 (11.8%) were older. For 7 rabbits (3.2%), the age was unknown (Table 2). Of the rabbits 115 were male (52.3%) and 105 female (47.7%). Due to an administrative error, there were 37 fanciers of whom we examined more than one rabbit (meaning two, three or four), instead of examining rabbits from 220 different rabbit breeders (Table 4).

3.2. Physical examination

All rabbits examined were alert and had a body condition score of at least 3 out of 5. Upon clinical examination, 8 rabbits had faeces matted in the fur of the perineum. None of the rabbits had diarrhoea at the moment of sampling.

3.3. Faecal analysis and physical examination

In total, 29 samples were discarded because there was not enough faeces to complete both a CSF analysis and a McMaster analysis.

3.3.1. CSF analysis

A CSF analysis was performed on 191 samples. Of these, 161 samples tested positive for *Eimeria* spp. (84.3%, 95% confidence interval (CI) 0.79 - 0.90) whereas 51 samples tested positive for *P. ambiguus* (26.7%, CI 0.20 - 0.33) (Table 3). *Cestoda* (species unidentified) and *Trichuris* spp. (most likely *T. leporis*) were each detected in 1 sample (0.5%, CI -0.01 - 0.02). Mixed infections with different *Eimeria* spp. were frequently observed, judged by the variations in shape and size of the oocysts. In one sample, at least two or three different species could often be identified.

3.3.2. McMaster analysis

A McMaster analysis was performed on the 169 samples that either tested positive for *Eimeria* spp. or for *P. ambiguus* or for both. In 49 samples (32.8%) that had tested positive for *Eimeria* spp. with the CSF analysis, the parasite could not be recovered with the McMaster analysis. Similarly, in 49 samples (22.9%) that were positive for *P. ambiguus* with the CSF analysis, no eggs were found with the McMaster. Therefore, no OPG or EPG could be calculated for these samples. *Eimeria* OPG (oocysts per gram faeces) ranged from 0 (which is, <100) to 111,400 (Median = 200) and *P. ambiguus* EPG (eggs per gram faeces) ranged from 0 (<100) to 400 (Median = 0.0) (Table 2). Using the McMaster method, *Cestoda* and *Trichuris* spp. could not be detected.

Table 1: Provinces of the Netherlands the rabbit fanciers that participated in the questionnaire were from. It was intended to randomly select rabbits from different rabbit fanciers and so, that the provinces were evenly distributed. Due to an administrative error, however, the provinces were not evenly represented. NH = Noord-Holland, ZH = Zuid-Holland, OV = Overijssel, GE = Gelderland, DR = Drenthe, BR = Noord-Brabant, GR = Groningen, FR = Friesland, LI = Limburg, UT = Utrecht, ZE = Zeeland, FL = Flevoland

Province	**NH**	**ZH**	**OV**	**GE**	**DR**	**BR**	**GR**	**FR**	**LI**	**UT**	**ZE**	**FL**
Number of rabbits	21	19	48	42	13	11	21	31	3	9	0	2

Table 2: Age of the rabbits in the study population. The OPG of Eimeria spp. and the EPG of P. ambiguus is displayed as range (median) for that age group. The highest values for OPG and EPG could be found in the rabbits that were 1 year old or younger, but it should be noted that this age group was better represented in the study population since the vast majority of rabbits was 1 year old or younger (n = 187, 85.0%).

Age (years)	**Number of rabbits (%)**	***Eimeria* OPG range (median)**	***P. ambiguus* EPG range (median)**
1	187 (85)	0 – 111400 (200)	0 – 400 (0)
2	17 (7.7)	0 – 4100 (100)	0 – 100 (0)
3	4 (1.8)	0 – 1200 (1150)	0 – 100 (0)
4	1 (0.5)	x	x
5	3 (1.4)	0 – 200 (100)	0 – 100 (0)
6	0	x	x
7	1 (0.5)	0 – 100 (0)	0 – 100 (0)
Unknown	7 (3.2)	x	x

Table 3: Prevalence of endoparasites in faeces of 191 Dutch show rabbits, as detected by CSF-analysis, with confidence intervals. Eimeria spp. were the most prevalent, followed by P. ambiguus. Cestodae and Trichuris spp. were each found in one faecal sample.

Endoparasite	**Prevalence**	**95% confidence interval**
Eimeria spp.	84.3%	0.79 - 0.90
P. ambiguus	26.7%	0.20 - 0.33
Cestodae	0.5%	-0.01 - 0.02
Trichuris spp.	0.5%	-0.01 - 0.02

3.4. Questionnaire results

A total of 173 survey forms was sent out, since not all 220 rabbits in the study population originated from different rabbit fanciers, as previously described. The questionnaire was answered by 83 of the rabbit fanciers, resulting in a response rate of 48%. Additional data on the number of rabbits and rabbit fanciers can be found in Table 4.

Table 4: Response to the questionnaire. All of the rabbit fanciers of whom we examined at least one rabbit were sent a survey form. There were 220 rabbits included in the study, owned by 173 rabbit fanciers, meaning that 173 survey forms were sent out. The questionnaire was answered by 83 rabbit fanciers.

Number of animals included in study	Number of rabbit fanciers	Number that responded to survey
1	183	68
2	29	12
3	6	2
4	2	1

In a number of cases, questions may have been misinterpreted by the rabbit fanciers. For example, on some occasions, they would both pick ‘No symptoms noted’ and ‘Diarrhoea noted’ when asked whether they had seen any clinical signs among their rabbits over the past year. In these cases, the ‘no symptoms noted’ was disregarded and the symptoms that were picked were recorded. Some rabbit fanciers would also specify age groups of affected rabbits for different symptoms, even though they had selected the option ‘No symptoms noted’. In these cases, the age groups were still collected in the data list.

Table 5: Data from the questionnaire. Dutch rabbit fanciers were asked a number of questions regarding the husbandry of their animals. Categorical variables are displayed as Category: frequency (percentage). Binary variables are displayed as Frequency (percentage). Of the rabbit fanciers that said to keep their rabbits in cages with wire mesh floors, 2 specified that they sometimes keep them in this kind of cages. These were still counted as 'yes'.

Number of rabbits	1-10: n = 20 (24.1%)	11-25: n = 40 (48.2%)	26-50: n = 22 (26.5%)	51-100: n = 1 (1.2%)
Number of litters/year	1-10: n = 40 (48.2%)	11-20: n = 35 (42.2%)	21-30: n = 7 (8.4%)	41-50: n = 1 (1.2%)
New rabbits/year	1-5: n = 78 (98.7%)	6-15: n = 1 (1.3%)		
Origin of new rabbits	Own breeding: n = 66 (79.5%)	Breeders in NL: n = 54 (65.1%)	Breeders in Europe: n = 21 (25.3%)	
Number of shows/year	1: n = 1 (1.2%)	2-4: n = 40 (48.2%)	>5: n = 42 (50.6%)	

	Yes	**No**	
Quarantine new rabbits	n = 21 (25.6%)	n = 61 (74.4%)	
Quarantine after show	n = 8 (9.6%)	n = 75 (90.4%)	
Individual housing	n = 77 (92.8%)	n = 1 (1.2%)	Both: n = 4 (4.8%)
Wire mesh floor in cage	n = 4 (4.9%)	n = 78 (95.1%)	
Clean cage when new rabbit	n = 75 (91.5%)	n = 7 (8.5%)	
Feed pellets with coccidiostat	n = 37 (48.1%)	n = 40 (52.0%)	
	Once a week	**Less than once a week**	
Clean cage	n = 71 (85.5%)	n = 10 (12.1%)	

Table 6: Cleaning agents and disinfectants used by the rabbit fanciers when cleaning the rabbits' cages. Note that due to an error in the questionnaire, the same product could be classified as a 'cleaning agent' by a number of rabbit fanciers, and as a 'disinfectant' by another. Therefore, no good distinction could be made between cleaning agents and disinfectants.

Use of a cleaning agent	n = 35 (35.7%)
Use of a disinfectant	n = 58 (63.7%)
Products used	**Frequency**
Soap/all-purpose cleaner	7
Vinegar	2
Dettol	11
Dasty	2
Chlorine	17
Halamid	13
Citronella	1
Tricel	1
Formalin	1
Gas burner	2
Steam	2

Husbandry

The majority of rabbit fanciers in the Netherlands owns less than 50 rabbits (n = 82, 98.8%), and about half of that group (n = 40, 48.2%) reports to have 11-25 rabbits (Table 5). Approximately one half of the rabbit fanciers breeds 1-10 litters each year (n = 40, 48.2%), and the other half 11-20 (n = 35, 42.2%). Then some of the breeders have more than 20 litters per year (n = 8, 9.6%). In most cases (78, 98.7%), 1-5 new rabbits are added to the stock each year, and most of these originate from their own litters (n = 66, 79.5%). Other sources for new rabbits are other rabbit breeders in the Netherlands (n = 54, 65.1%) or in Europe (n = 21, 25.3%). When adding a new rabbit to the stock, about a quarter of the rabbit fanciers (n = 21, 25.6%) take quarantine measures. About half of the rabbit fanciers (n = 40, 48.2%) attend 2-4 shows with their animals during the showing season each year and the other half attends more than 5 shows each season (n = 42, 50.6%). After visiting one of these shows, most of them (n = 75, 90.4%) do not take quarantine measures for their animals.

Most of the rabbit fanciers (77; 92.8%) house their rabbits individually and the majority (n = 78, 95.1%) keeps their animals in cages with a solid floor and bedding. When it comes to cage hygiene, most of the breeders (n = 70, 84.3%) clean the cages once a week or more and 10 (12.1%) clean them less than once a week. When new animals are added, the majority of rabbit fanciers (n = 75, 91.5%) clean their cages first. Some of them indicate that they use a cleaning agent (n = 35, 35.7%) or disinfectant (n = 58, 63.7%) when cleaning the cages of the rabbits. Products used can be found in table 6. It should be noted that the same products would be classified by some respondents as a cleaning agent and by others as disinfectant. About half of the breeders (37; 48.1%) feed their rabbits pellets that contain a coccidiostat, in most of the cases robenidine hydrochloride.

Table 7: Clinical signs, possibly related to endoparasite infestation, that were noted by the responding rabbit fanciers in their stock over the past year, displayed as number of rabbit fanciers that noted the symptom in their rabbits followed by percentage.

Clinical sign	**n (%)**
Diarrhoea	28 (33.7)
Anorexia	24 (28.9)
Distended abdomen	20 (24.1)
Reduced growth in young rabbits	12 (14.5)
Significant mortality	12 (14.8)
Weight loss	10 (12.1)
Disappointing breeding results	10 (12.1)

Clinical signs
About half of all respondents (n = 36, 43.4%) have not noticed clinical signs possibly related to endoparasite infection in their rabbits over the past year. The clinical signs noted by the other half of the rabbit fanciers can be found in table 7. The mean number of animals that was affected in these cases was 7.39 (33 respondents), and the mean number of animals that died when significant mortality was noticed was 6.6. Rabbit fanciers were asked to specify which age group was most affected for each clinical sign they noted (Figure 1). When significant mortality was noted, in most of the cases (n = 22, 91.7%) the cause of the mortality remains unknown. In 2 cases (8.3%), the cause of the mortality was confirmed by a veterinarian, being Rabbit Haemorrhagic Disease Virus (RHDV) in one case and a period of heat in the other. It should be noted that only 12 rabbit fanciers said they had seen notable mortality among their animals, but 17 specified which age group was affected and 24 indicated whether the cause was determined by a veterinarian.

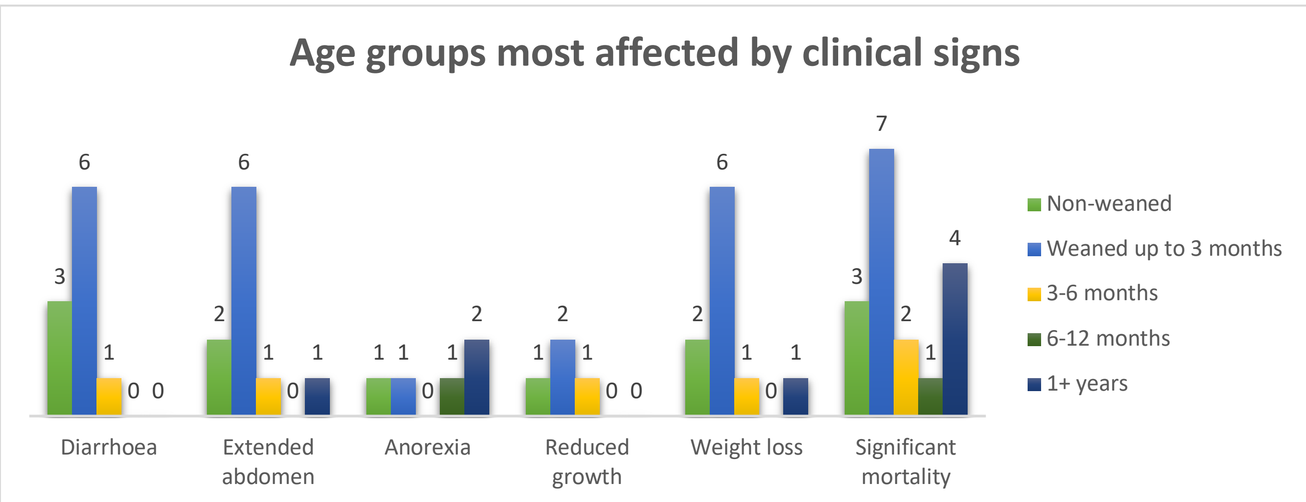

Fig. 1: Age groups of rabbits that were most affected by clinical signs possibly related to endoparasite infestation, as recorded by the rabbit fanciers, displayed as number of rabbits per clinical sign.

Rabbit fanciers were asked whether their animals were diagnosed with coccidiosis by a vet over the past year. For most fanciers, this was not the case. Rabbits of 2 (2.4%) breeders were diagnosed with coccidiosis. Treatment was implemented in one of these cases, in the other case, the infection disappeared spontaneously. The majority of the rabbit fanciers did not treat their rabbits for worms over the past year (n = 74, 90.2%).

3.5. Risk factor analysis

By extrapolating the responses of the breeders of whom multiple animals were examined to these additional rabbits, we acquired data for 104 rabbits for the questionnaire. The dataset acquired this way was used for the risk factor analysis only. A risk factor analysis was performed to detect a possible association between the risk factors for disease and the presence of *Eimeria* spp. or *P. ambiguus* and the *Eimeria* OPG. For the risk factor analysis the data of 88 rabbits were used, namely those rabbits of which we had both obtained results from the faecal analysis and from the questionnaire. Data were collected for a total of 25 variables. A number of variables were excluded from the risk factor analysis because of a lack of variation. These can be found in Appendix IV, together with all *p*-values for the Pearson's Chi-square test. After this, 14 variables remained. Some observations had missing data. The percentages of missing data for the variables can be found in Appendix V. Observations with missing data were removed in order to perform a multivariable logistic regression. The multivariable logistic regression for the outcome variables *Eimeria* and *Passalurus* were performed with 63 observations and 3 and 4 variables respectively. To perform the multivariable linear regression for *Eimeria OPG,* some additional observations with missing data for the OPG needed to be removed; namely the 9 observations where we did not perform a McMaster analysis because the CSF-analysis was negative for all endoparasites. Therefore, this linear regression was performed with 54 observations and 2 variables.

The final model for *Eimeria* spp. presence consisted of 3 variables: Whether quarantine is implemented for new rabbits, whether the rabbit fancier uses a cleaning agent when cleaning the cages and whether the rabbit fancier noticed a distended abdomen in his/her rabbits over the past year. Of these parameters, only quarantine was statistically significant ($p = 0.007$). There was a trend toward significance in the noting of a distended abdomen ($p = 0.10$). P-values and 95% confidence intervals can be found in Table 8.

The final model for *P. ambiguus* presence consisted of the following 4 variables: Whether quarantine is implemented for new rabbits, whether the rabbits are fed pellets containing a coccidiostat, whether a disinfectant is used when cleaning the cages and whether reduced growth was noted among the rabbits over the past year. Quarantine ($p = 0.02$) was found to have a statistically significant influence on identifying *P. ambiguus* in the faeces (Table 9).

The final linear regression model for the *Eimeria* spp. OPG consisted of two variables: Whether the rabbit fancier implements quarantine for new rabbits and whether he/she uses a cleaning agent when cleaning the hutches of the rabbits. Of these, the use of a cleaning agent was statistically significant (*p* = 0.01) (Table 10).

Table 8: Risk factor analysis for Eimeria spp. presence in faeces from Dutch rabbit fanciers: Odds ratio (OR), 95% confidence interval (95% CI) and p-values for the final Eimeria multivariable logistic regression model. AIC values were calculated for the variables with p < 0.25 as determined by Pearson's Chi-square test, and the following factors contributed to the model fit (That is, they did not increase the AIC value): Quar = quarantine when new rabbits are acquired, CleanAg = use of a cleaning agent when cleaning the cages, DistAbd = noticing of a distended abdomen in the rabbits.

Variable	*Eimeria* positives (%)	*Eimeria* negatives (%)	OR	95% CI	*p*-value
Quar					
No	41 (65)	5 (7.9)	0.17	-3.94 to -0.69	0.007
Yes	10 (16)	7 (11)			
CleanAg					
No	32 (51)	8 (13)	1.2	-0.36 to 3.02	0.16
Yes	19 (30)	4 (6.3)			
DistAbd					
No	35 (56)	11 (17)	5.0	0.02 to 4.97	0.10
Yes	16 (25)	1 (1.6)			

Table 9: Risk factor analysis for P. ambiguus presence in faeces from Dutch rabbit fanciers: Odds ratio (OR), 95% confidence interval (95% CI) and p-values for the final P. ambiguus multivariable logistic regression model. AIC values were calculated for the variables with p < 0.25 as determined by Pearson's Chi-square test, and the following factors contributed to the model fit (That is, they did not increase the AIC value): Quar = quarantine when new rabbits are acquired; coccidiostat in feed, use of a disinfectant, and reduced growth in young rabbits.

Variable	*P. ambiguus* positives (%)	*P. ambiguus* negatives (%)	OR	95% CI	*p*-value
Quar					
No	14 (22)	32 (51)	0.14	-5.63 to -0.76	0.02
Yes	1 (1.6)	16 (25)			
Coccidiostat					
No	10 (16)	22 (35)	0.42	-2.29 to 0.47	0.22
Yes	5 (7.9)	26 (41)			
Use of a disinfectant					
No	5 (7.9)	20 (32)	1.43	-0.29 to 2.47	0.14
Yes	10 (16)	28 (44)			
Reduced growth					
No	14 (22)	37 (59)	0.24	-4.53 to 0.50	0.20
Yes	1 (1.6)	11 (17)			

Table 10: Risk factor analysis for Eimeria spp. OPG in faeces from Dutch rabbit fanciers: Odds ratio (OR), 95% confidence interval (95% CI) and p-values for the final Eimeria OPG multivariable linear regression model. AIC values were calculated for the variables with p < 0.25 as determined by Pearson's Chi-square test, and the following factors contributed to the model fit (That is, they did not increase the AIC value): Quar = quarantine when new rabbits are acquired, CleanAg = use of a cleaning agent when cleaning the cages.

Variable	OR	95% CI	*p*-value
Quar	0.66	-0.99 to 0.11	0.15
CleanAg	1.75	0.14 to 0.97	0.01

4. Discussion

4.1. Prevalence

The aim of this study was to determine the prevalence of *Eimeria* species, *P. ambiguus* and other endoparasites in rabbits from Dutch rabbit fanciers; and to determine to what extent these endoparasites are associated with onset of clinical disease, including the risk factors associated with it. The prevalence of *Eimeria* spp. was 84.3% and that of *P. ambiguus* 26.7%; other endoparasites found were *Cestoda* and *Trichuris* spp., both with a prevalence of 0.5%.

4.1.1. *Eimeria* spp.

The prevalence of *Eimeria* spp. (84.3%) was higher than hypothesised. This prevalence is similar to that found in Dutch rabbit shelters, which was 72% and 89% in official and private shelters, respectively (Hulsinga, 2020). Which *Eimeria* species were found, was not determined in this study. The high prevalence combined with a low frequency of clinical signs suggests that infection with *Eimeria* species of a low pathogenicity is common in these rabbits (Lebas et al, 1997).

4.1.2. P. ambiguus

The prevalence of *P. ambiguus* (26.7%) was much higher than hypothesised. Other studies found this nematode to be present in 3-5.7% of rabbits (Mäkitaipale et al, 2017; Raue et al, 2017; Sürsal et al, 2014; Elshahawy & Elgoniemy, 2018). A possible explanation for the high prevalence in the present study could be that the prevalence was based on the CSF-analysis, compared to other studies that solely used a McMaster analysis (Mäkitaipale et al, 2017). However, other studies also used a sedimentation-flotation protocol (Raue et al, 2017; Sürsal et al, 2014; Elshahawy & Elgoniemy, 2018). In a study that compared the CSF-analysis, passive flotation and the McMaster method for examination of dog faeces, no significant difference was found between CSF-analysis and McMaster method, although the McMaster method led to less positive tests (Somers, 2015). One study did show the FLOTAC technique was more sensitive than the McMaster method (Rinaldi et al, 2007). Another study comparing coprological methods in veterinary diagnostics showed that the McMaster method had a reliable sensitivity at a minimum of 500 EPG, while that of combined sedimentation-flotation was reliable at lower EPG levels, depending on the parasite species and flotation medium. This study pointed out, however, that results regarding sensitivity and efficiency of a test obtained for one parasite species cannot be readily extrapolated to other parasite species (Becker et al, 2016). Therefore, research comparing the CSF-analysis and McMaster method for *P. ambiguus* is necessary to know whether this could have influenced the prevalence found in the present study.

In the present study, the faecal samples were all collected during the late afternoon or evening hours, which could contribute to the high prevalence as well. It is known that eggs of *P. ambiguus* are shed in a circadian rhythm, and that sampling during the afternoon and night hours yields the best chance of detecting the parasite (Rinaldi et al, 2007). For the other studies mentioned, the time of sampling was not recorded. This could mean that the prevalence of *P. ambiguus* has been underestimated in previous research.

4.1.3. Other endoparasite species

Next to *Eimeria* spp. and *P. ambiguus*, *Cestoda* and *Trichuris* spp. were both found in one sample (0.5%). *Trichuris* spp. are not frequently reported in pet rabbits. A study of 434 samples of rabbit faeces, collected from pet rabbits in Germany over a period of 10 years, reported a prevalence of 0.2% (Raue et al, 2017). Another report found a prevalence of 0.25% for both *Cestoda* and *T. leporis (*Mäkitaipale et al, 2017*)*.

4.2. *Eimeria* OPG

The OPG varied greatly between rabbits. It ranged from <100 to 111,400 with a median of 200 and a mean of 2385. A wide range in OPG with a median and mean in the lower part of the range is consistent with the literature. A study among pet rabbits in Finland found similar results, with a range of 25-142,500; a median of 263 and a mean of 4212 (Mäkitaipale et al, 2017). In Dutch official rabbit shelters, the OPG ranged from 0 to 25550, with a median of 500 and a mean of 1938. In private shelters it ranged from 0 to 12150, with a median of 1000 and a mean of 7558 (Hulsinga, 2020). The different species of *Eimeria* present in the faecal samples were not differentiated because time for this study was limited. It would be interesting for future studies to determine which species are present and to connect this to possible risk factors.

4.3. Clinical signs

A total of 47 (56.6%) rabbit fanciers noticed one or more of the clinical signs possibly related to either *Eimeria* spp. or *P. ambiguus* infection - diarrhoea, anorexia, a distended abdomen, reduced growth, weight loss or disappointing breeding results – in their rabbits over the past year.

4.3.3. *Eimeria* spp.

There was a trend towards significance for noticing a distended abdomen in rabbits and the presence of *Eimeria* spp. in the faeces. The odds ratio was 5.0 (p = 0.10), but it should be noted that there were fewer non-cases than cases for the *Eimeria* spp. risk factor analysis. A distended abdomen is not frequently mentioned as a sign of coccidiosis in the rabbit, although it has been recognised in a case report (Kala et al, 2019). It has been observed in rabbits with hepatic coccidiosis as well (Çam et al, 2008). A distended abdomen, together with diarrhoea, inappetence and sudden death, was associated with coccidiosis by rabbit farmers in Central Kenya (Ogolla et al, 2017). However, a distended abdomen can be found with numerous other diseases of rabbits, such as bloating and epizootic rabbit enteropathy (Licois et al, 2005). No association could be found between the other clinical signs and the risk factors for disease. The clinical signs included in the questionnaire are all not specific for coccidiosis. Symptoms could have been missed as well. For example, weight loss or reduced growth are not easily or routinely measured and could be overlooked. Additionally, the questionnaire results could be less reliable due to the fact that some questions may have been misinterpreted by the rabbit fanciers, as indicated by the ambiguous answers. These observations could indicate that the filled out results are not reliable, which therefore means it remains uncertain whether a correlation between the clinical signs and the presence and OPG of *Eimeria* spp is or is not present.

4.3.2. *Eimeria* OPG

Despite the finding of some very high OPG values, no rabbits showed any clinical signs at the moment of sampling. Therefore, it can be concluded that a high *Eimeria* OPG does not necessarily lead to clinical disease. Similar results were obtained by Lohkamp (2020). The OPG is dependent both on housing and feeding management and on the immune status of the individual rabbit (Lohkamp, 2020). Under identical experimental conditions, the number of oocysts excreted by infected rabbits varies greatly (Ryley & Robinson, 1976; Pakandl, 2013). Rabbits are often infected at a very young age and build immunity against the species of coccidia that they encounter. The degree of immunity depends on the species: some *Eimeria* species induce a longer lasting immunity, whereas others can still reproduce to some extent upon re-infection. Older animals usually shed lower numbers of oocysts in their faeces, although these numbers may rise when the animal is weakened, e.g. by another disease (Lohkamp, 2020). This study, however, did not find an association between the age of the rabbits and the *Eimeria* OPG. The age variable was excluded from the regression model as the *p*-value in Pearson's Chi-square test was >0.25. It should be noted that 187 of the rabbits we examined were 1 year old or younger and only 26 animals were older than 1 year. This could account for the fact that no association was found between age and OPG. Further research is needed on this topic, where equal numbers of rabbits in different age groups need to be sampled.

4.3.3. *P. ambiguus*

The rabbit pinworm *P. ambiguus* is generally thought to be non-pathogenic (Varga, 2013), although it has been linked to poor breeding performance and poor condition (Düwel & Brech, 1981). Recently, a study found chronic catarrhal colitis in rabbits with passaluris load (Mykhailiutenko et al, 2019). In the present study, both the factors of weight loss and disappointing breeding results had to be excluded from the risk factor analysis because of a lack of variation in survey results. Therefore, a possible correlation to the presence of either parasite could not be investigated.

4.4. Risk factors

This study found an association between the presence of both *Eimeria* spp. and *P. ambiguus* and the implementing of quarantine measures when the rabbit fanciers add new rabbits to their stock. Odds ratios were 0.17 (p = 0.007) and 0.14 (p = 0.02), respectively. This indicates that implementing quarantine measures is important in preventing *Eimeria* spp. and *P. ambiguus* infection. A possible explanation could be that the stress of changing environments leads to the rabbits excreting higher numbers of infective oocysts, resulting in higher parasitic load of the environment. Pregnant and lactating rabbits were found to have peaks of oocyst excretion after parturition and during lactation. This was most likely due to an activation of latent *Eimeria* infection, possibly caused by reduced resistance to infections by the stress and hormonal changes during these events (Papeschi et al, 2013).

About half of the respondents (37; 48.05%) to the questionnaire feed their rabbits pellets that contain anticoccidia. In most of the cases (30; 81.08%), the coccidiostat present is robenidine hydrochloride. Propyl gallate and buthylhydroxytoluene, both antioxidants, are mentioned by one breeder. However, no scientific evidence on the effects of these antioxidants on coccidiosis in rabbits was found. Robenidine hydrochloride, administered in the feed, prevented a reduction in growth in young rabbits challenged with *Eimeria* spp. and significantly lowered the oocyst excretion (Vancraeynest et al, 2008). This study found no correlation between feeding pellets that contain a coccidiostat and the presence of *Eimeria* spp. and *P. ambiguus* or the *Eimeria* OPG. This may indicate that the feeding of pellets containing a coccidiostat is not necessary for rabbits from rabbit fanciers. Resistance against robenidine hydrochloride has already been found in strains of *E. magna* and *E. media* (Peeters, 1988; Coudert, 2004). Further research on this topic is necessary, to acquire insight in the different species of *Eimeria* in this sector of the Dutch pet rabbit industry and potential resistance to coccidiostats in rabbit feed.

Although infection with high numbers of *Eimeria* oocysts does not necessarily lead to clinical disease, coccidia can facilitate secondary infections with bacteria (Lohkamp, 2020). It is therefore necessary to limit the number of oocysts newborn and newly weaned rabbits are exposed to by good hygienic measures. The way the rabbits are kept is of influence to the infection pressure as well: A lower number of animals in a certain space means a lower parasitic load, as well as keeping the rabbits in cages with wire mesh floors (Lohkamp, 2020). The latter is, however, not preferred because of the risk of developing ulcerative pododermatitis (Rosell et al, 2013). Since most rabbit fanciers in this study do not keep their rabbits in cages with wire mesh flooring (80 do not; 97.6%), the effects of this factor could not be studied. Cage hygiene was found to be an important risk factor for pathogenic *Eimeria* infection in a recent study in animal shelters in the Netherlands (Hulsinga, 2020). Most of the respondents (70; 84.34%) mentioned to clean the cages of their rabbits once a week or more frequently, so this factor had to be excluded from the risk factor analysis as well. The present study found an association between the *Eimeria* OPG and the use of a cleaning agent when cleaning the rabbit's cages. The odds ratio was 1.75 (p = 0.01), pointing towards a rise in OPG when using a cleaning agent. This is in line with the results of a recent study in Dutch pet shops, which found a significantly higher OPG when the disinfectant Halamid® was used (Van der Plas, 2020). This effect could possibly be explained by the fact that using a cleaning agent or disinfectant could give the user a secure feeling, leading to them less frequently or thoroughly cleaning the cages. Halamid® was used by 13 respondents out of 93 (14.0%) that used either a cleaning agent or disinfectant or both. No analysis was done, however, on the type of cleaning agent used, because of the high diversity in products used by the rabbit fanciers. On multiple occasions, the participants in the questionnaire would state that they use a cleaning agent or disinfectant, but when asked to define which product they use, they would pick the option 'I don't use a cleaning agent or disinfectant'. This makes the results for these factors less reliable. Further research is needed on this topic, with randomization of cleaning products used.

All respondents to the questionnaire participate in at least 2 rabbit shows each year. Of these, 42% even attend 5 shows or more. Attending rabbit shows could be a stress factor for the rabbits. Most rabbits that are showed are 1 year old or younger and large numbers of rabbits are present at these shows. These things combined could predispose for a clinical *Eimeria* infection. This study, however, found no correlation between the number of shows that are attended annually and the presence of *Eimeria* spp. or *P. ambiguus* presence. At the show, all rabbits were housed individually, animals were not mixed or moved. This could prevent spreading of parasite infections, thus contributing to the lack of correlation found in this study. Whether or not the rabbit fanciers implemented some form of quarantine after visiting a show was also not associated with the presence of either parasite or with *Eimeria* OPG.

4.5. Limitations

This study had some limitations, which will be described below.

4.5.1. Faecal examination

29 faecal samples had to be discarded because there was not enough faeces to complete both a CSF-analysis and the McMaster method. This did not lessen the reliability of the study, however, since the power analysis showed that 160 rabbits needed to be sampled, and there were still 191 samples left. Still, the accuracy of our faecal diagnostic tests was lessened by only counting one counting chamber of the McMaster slide and by leaving out the McMaster method if the CSF-analysis was negative. Studies have shown that the reliability of the McMaster method is dependent, among other things, on the volume of the slide that is counted: When a smaller area is counted with a high level of infection, egg counts tend to be higher (Bosco et al, 2014; Cringoli et al, 2004). By leaving out the McMaster method if the CSF-analysis was negative, it is possible that the prevalence of the endoparasites was underestimated because some infections may have showed with the McMaster method and not with the CSF-analysis.

The samples were stored in a refrigerator. Because it took about ten weeks to process all samples, part of the faecal material was stored for several weeks. A study on storage factors influencing egg count of *Strongylides* in horses showed that, when kept in a refrigerator, faecal samples showed no decline in egg count for op to 120 hours of storage (Nielsen et al, 2010). It is not known, however, what the effect of storing samples for ten weeks on egg count is. It is possible that egg and oocyst counts and prevalence were underestimated as a result of this, but no difference in results was noted by the author during faecal examination: The results seemed to be consistent during all weeks of the examination.

4.5.2. Questionnaire

Regarding the questionnaire, there was little variation in the answers for some of the variables. These variables can be found in Appendix IV. From this, it can be concluded that rabbits from Dutch rabbit fanciers are kept in fairly uniform conditions. For this reason, the importance of these risk factors could not be determined, and these variables were excluded from the risk factor analysis. Additionally, data about the risk factors were obtained from the rabbit fanciers, who are not trained observers. As mentioned before, various answers suggested that some questions were misinterpreted, making the questionnaire less reliable. It should be kept in mind that socially desirable answers could be given by the rabbit fanciers. Studies have shown that questionnaires are not always reliable for research. (Gilbody et al, 2001; Drewnowski, 2001)

4.5.3. General

Furthermore, a sample of rabbits at a rabbit show might not be very representable for the population of rabbits that are kept and bred as part of a hobby in the Netherlands. The rabbits that are brought to a show are most often young animals below one year of age. In our case, 85.0% of the rabbits we selected for the study were one year old or younger.

Additionally, only rabbits that are healthy and in a good condition are brought to shows. This may have resulted in a selection bias. These notes should be kept in mind when interpreting the results of this study.

4.6. Implications

This study showed that *Eimeria* spp. presence is very common in rabbits from Dutch rabbit fanciers and that the prevalence of *P. ambiguus* is much higher than commonly expected. It also showed that implementing quarantine measures when new rabbits are acquired significantly reduced the likelihood of *Eimeria* spp. and *P. ambiguus* being present in faeces. Therefore, rabbit fanciers are recommended to apply quarantine measures when they want to acquire new rabbits. Additional studies are needed to specify what kind of quarantine measures this should be, as this was not investigated. This study also showed that the *Eimeria* OPG was significantly higher if the rabbit fancier uses a cleaning agent when cleaning the rabbits' cages. It could possibly be explained by a secure feeling the use of a product gives the rabbit fancier, leading to less frequent or thorough cleaning. This requires additional research as well, controlled studies with randomizing of cleaning products used, to confirm and explain this phenomenon.

5. Conclusion

The prevalence of *Eimeria* spp. among rabbits from Dutch rabbit fanciers was found to be 84.3% and that of *P. ambiguus* was 26.7%. In addition, *Cestodae* and *Trichuris* spp. were both prevalent in 0.5% of rabbits. Implementation of quarantine measures when new rabbits are acquired reduced the likelihood of *Eimeria* spp. and *P. ambiguus* being present in the faeces, indicating the importance of this measure for the prevention of endoparasite infection. Additionally, the Eimeria OPG was significantly higher if a cleaning agent was used when cleaning the rabbits' cages. This needs to be further studied.

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Appendix I: Clinical examination form

A. General impression	A.1. Level of consciousness: sopor/stupor A.2. Body condition score (on a scale of 1-5; 3 = normal) A.3. Condition of fur and nails A.4. Notable clinical signs
B. Skin, fur, nails	B.1. Skin lesions: specify type, location and extension B.2. Fur: broken hairs/baldness/scales/other, namely: B.3. Nails: crumbled/overgrown B.4. Other signs
C. Ears	C.1. Auricle: redness/swelling/crusts C.2. Ear canal: excessive cerumen/pus/ear mites C.3. Other signs
D. Eyes	D.1. Discharge: serous/seromucoid/mucoid/mucopurulent/ purulent D.2. Eyelids: blepharospasm/swelling/crusts/lesions (Describe if present) D.3. Mucous membranes: red/swollen/other
E. Nose	E.1. Lesions: Describe if present E.2. Secretions: serous/seromucoid/mucoid/mucopurulent/ purulent - Unilateral/bilateral
F. Teeth	F.1. Malocclusion: Describe if present
G. Perianal area	G.1. Faeces stuck in fur/redness/swelling/other
H. Feces	H.1. Small faecal pellets/diarrhoea/admixtures (Describe if present)

I. Other clinical signs	I.1 Describe if present

Appendix II: Faecal analysis protocols

CSF analysis

A qualitative method of determining the presence of endoparasite eggs or oocysts in faeces.

1. Make a suspension of the faecal sample in tap water. Use a pestle to grind hard faecal pellets. Make sure not to use too much or too little water, about 20 milliliters for each tea spoon full of faeces.
2. Pour the suspension over a sieve in a cup.
3. Stir the suspension, because the eggs are on the bottom of the cup. Fill a centrifuge tube with the suspension.
4. Place the tube in the centrifuge and spin for 2 minutes at 3000 revs/minute. Always make sure the centrifuge is balanced by placing 2 tubes facing each other.
5. Slowly turn the tube upside down and let the supernatant flow away.
6. Fill the tube halfway with a sugar solution and bring the sediment in suspension using a vortex.
7. Put the tube back in the centrifuge and fill it up with the sugar solution until there is a small positive meniscus above the rim of the tube.
8. Put a cover slip on the meniscus and slightly press with a fingernail.
9. Spin the tube in the centrifuge, the same way as in step 4.
10. Take the cover slip off the tube and put it on an object glass.
11. Systematically search the slide for eggs or oocysts under the microscope.

McMaster analysis

A quantitative method of determining the presence of endoparasite eggs or oocysts in faeces and their EPG (eggs per gram faeces) or OPG (oocysts per gram faeces).

1. Weigh 3 grams of faeces in a 50 ml Falcon tube. Use a pestle to grind hard faecal pellets.
2. Put 42 milliliters of a saturated salt solution in another Falcon tube.
3. Add a small amount of this solution to the faeces and make a suspension with the pestle.
4. Pour the suspension over a sieve into a cup, followed by the remainder of the 42 mililiters of saturated salt solution.
5. Pour the contents of the cup back into the 50 ml Falcon tube.
6. Close the tube and swerve approximately 10 times, don't shake the tube.
7. Remove the cap and fill the first compartment McMaster counting chamber.
8. Close the tube and swerve again before filling the second compartment.
9. Wait 1 minute before counting the eggs under the microscope, using the x10 objective.
10. The OPG or EPG is the number of oocysts or eggs multiplied by 50 (Or in our case, by 100, since we only counted one compartment of the McMaster counting chamber).

Appendix III: Questionnaire

General part

1. How many rabbits did you own at the time of the Noordshow 2020?
    a. 1-10 rabbits
    b. 11-25 rabbits
    c. 26-50 rabbits
    d. 51-100 rabbits
    e. > 100 rabbits
2. How many litters do you breed per year, on average?
    a. 1-10 litters
    b. 11-20 litters
    c. 21-30 litters
    d. 31- 40 litters
    e. 41-50 litters
3. How many new rabbits do you acquire each year from other sources (e.g. other rabbit fanciers, pet trade, pet shops)
    a. 1-5 rabbits
    b. 6-15 rabbits
    c. 16-30 rabbits
    d. 31-50 rabbits
    e. < 50 rabbits
4. What sources do your rabbits come from? S*elect all applicable options and give an indication of the percentage of rabbits that came from each source in question 5.*
    a. From own litters
    b. From another Dutch rabbit fancier
    c. From another rabbit fancier from Europe
    d. From another rabbit fancier from outside of Europe
    e. From pet trade
    f. From a pet store
    g. From an animal shelter or rescue
    h. Other, namely:
5. Indicate the percentages of your rabbits that came from the sources you selected in question 4.
6. When acquiring new rabbits, do you implement quarantine measures? If so, what are these measures?
    a. No
    b. Yes, namely:
7. How many rabbits are removed from your population each year, on average?
    a. 1-5 rabbits

    b. 6-15 rabbits
    c. 16-30 rabbits
    d. 31-50 rabbits
    e. > 50 rabbits
8. Where do these rabbits that are removed from the population go to? *Select all applicable options and give an indication of the percentages of rabbits that goes there.*
    a. Another Dutch rabbit fancier
    b. Another rabbit fancier in Europe
    c. Another rabbit fancier outside of Europe
    d. Sold as a pet to Dutch owners directly
    e. Sold as a pet to people in other European countries directly
    f. Sold as a pet to people in countries outside Europe directly
    g. Pet trade, to be sold as a pet
    h. Pet trade, to be sold as a feed animal
    i. Pet trade, to be sold as breeding stock
    j. Pet trade, other
    k. Pet shop
    l. Animal shelter or rescue
    m. Other, namely:
9. Indicate the percentages of rabbits that go to the options you chose in question 8.
10. How often do you take your rabbits to rabbit shows, exhibitions, and fairs each year, on average?
    a. Once per season
    b. 2-4 times per season
    c. More than 5 times per season
11. When you have taken your rabbits to a rabbit show, exhibition, or fair, do you implement quarantine measures?
    a. No
    b. Yes, namely:
12. How do you house the majority of your rabbits?
    a. Individual housing (1 rabbit per cage)
    b. Housing in pairs (2 rabbits per cage)
    c. Group housing (more than 2 rabbits per cage)
    d. Other, namely:
13. If you selected group housing, how many rabbits do you house in one cage, on average?
    a. 2-5 rabbits per cage
    b. 6-10 rabbits per cage
    c. 11-15 rabbits per cage
    d. More than 15 rabbits per cage

14. What type of housing do you have for your rabbits?
    a. Cages with a wire mesh floor without bedding
    b. Cages with a wire mesh floor with bedding
    c. Cages with a solid floor with bedding
    d. Other, namely:
15. How often do you clean the cages?
    a. Twice a week
    b. Once a week
    c. Once every two weeks
    d. Other, namely:
16. Do you clean the cages before you house new rabbits in them?
    a. Yes
    b. No
17. If you selected 'Yes' for question 16, which cleaning product do you use?
    a. None, I only clean out the bedding
    b. Soap/all-purpose cleaner
    c. Vinegar
    d. Dettol
    e. Chlorine
    f. Water
    g. Other, namely:
18. If you selected 'Yes' for question 16, which disinfectant do you use?
    a. No disinfectant
    b. Dettol
    c. Chlorine
    d. Rolith
    e. Halamid
    f. Vinegar
    g. Soap/all-purpose cleaner
    h. Virkon S.
    i. Other, namely:
19. Which brand of pellets do you feed your rabbits?
20. Do these pellets contain a coccidiostat (see label on the food bags), e.g. robenidine (Cycostat) or diclazuril (Clinacox)?
    a. No
    b. Yes, namely:

Endoparasites

21. Did you notice one of the following symptoms in your rabbits over the past year? Select all applicable options.
    a. Diarrhoea

b. Distended abdomen
c. Weight loss
d. Anorexia
e. Reduced growth in young rabbits
f. Disappointing breeding results in bucks or does
g. No symptoms noted

22. When you have selected one or more of options a through f in question 21, how many of your rabbits showed these symptoms?

23. When you have selected one or more of options a through e in question 21, indicate the age group that was most affected for each of the symptoms:
    a. Diarrhoea
        a. Suckling rabbits
        b. Weaned rabbits up to 3 months of age
        c. Rabbits between 3 and 6 months of age
        d. Rabbits between 6 and 12 months of age
        e. Rabbits older than 1 year of age
    b. Distended abdomen
        a. Suckling rabbits
        b. Weaned rabbits up to 3 months of age
        c. Rabbits between 3 and 6 months of age
        d. Rabbits between 6 and 12 months of age
        e. Rabbits older than 1 year of age
    c. Weight loss
        a. Suckling rabbits
        b. Weaned rabbits up to 3 months of age
        c. Rabbits between 3 and 6 months of age
        d. Rabbits between 6 and 12 months of age
        e. Rabbits older than 1 year of age
    d. Anorexia
        a. Suckling rabbits
        b. Weaned rabbits up to 3 months of age
        c. Rabbits between 3 and 6 months of age
        d. Rabbits between 6 and 12 months of age
        e. Rabbits older than 1 year of age
    e. Reduced growth in young rabbits
        a. Suckling rabbits
        b. Weaned rabbits up to 3 months of age
        c. Rabbits between 3 and 6 months of age
        d. Rabbits between 6 and 12 months of age
        e. Rabbits older than 1 year of age

24. Did you notice significant mortality over the past year? If yes, how many rabbits died?
    a. No
    b. Yes, number:

25. If you selected ‘Yes’ for question 24, what age group was most affected by mortality?
    a. Suckling rabbits
    b. Weaned rabbits up to 3 months of age
    c. Rabbits between 3 and 6 months of age
    d. Rabbits between 6 and 12 months of age
    e. Rabbits older than 1 year of age

26. If you selected ‘Yes’ for question 24, was the cause of the mortality determined by a veterinarian?
    a. No
    b. Yes, the cause was:

27. Were any of your rabbits diagnosed with coccidiosis by a veterinarian over the past year?
    a. No
    b. Yes

28. If you selected ‘Yes’ for question 27, was a treatment implemented? If yes, what treatment (by feed, in drinking water, by injection)?
    a. No treatment was implemented, the infection passed on its own
    b. No treatment was implemented, the infection is still present
    c. Yes, treatment was implemented, namely:

29. Did you treat your rabbits against worms over the past year? If yes, what drug did you use?
    a. No
    b. Yes, the drug used was:

Appendix IV: Variables excluded from the models

Variables that were excluded from the model for a lack of dispersion.

Variable	Deviaton
New rabbits/year	98.7% adds 1-5 new rabbits
Quarantine after show	90.4% do not quarantine
Solitary housing	92.8% do house solitary
Wire mesh	97.6% do not keep rabbits on wire mesh
Clean cage <1 times/week	84.3% do not clean cages <1 times a week
Clean cage when new animals arrive	91.5% do clean when new animals arrive
Worm treatment	90.2% did not treat for worms
Coccidiosis confirmed	97.6% did not have a confirmed case
Weight loss	88.6% did not notice weight loss
Disappointing breeding	86.4% did not notice disappointing breeding
Significant mortality	85.2% did not notice significant mortality

Appendix V: Missing data

Variables with percentages of data that were missing. First, data from breeders that did not participate in the questionnaire were excluded from the original dataset including 220 rabbits. This way, 88 rabbits remained. After that, missing data were calculated. In order to do a logistical regression, the observations with missing data were removed, leaving a set of 63 observations and 12 variables.

Variable	Missing data (%)
Quarantine when new rabbits	2.3%
Use cleaning agent	4.5%
Use disinfectant	12.5%
Coccidiostat	13.6%
Reduced growth	1.1%

Appendix VI: *p*-values from the Pearson's Chi-squared test

p-values for the variables from the Pearson's Chi-squared test.
* Removed because of a lack of variation in answers

Variable	***p*-value (*Eimeria*)**	***p*-value (*Passalurus*)**	***p*-value (*Eimeria* OPG)**
Province	0,3959	0,8116	0,3959
Age of rabbit (years)	0,2007	0,7973	0,9998
Gender of rabbit	0,3295	0,6084	0,3611
Number of rabbits	0,7597	0,5434	0,05547
Number of litters/year	0,3907	0,7975	0,01955
*New rabbits/year**	0,6366	0,5211	1
Quarantine for new rabbits	0,01158	0,05567	0,8838
Five plus shows/year	0,7768	0,8131	0,6123
*Quarantine after show **	0,8564	0,6677	0,2698
*Solitary housing **	0,5251	0,5179	7,917 e-08
*Wire mesh flooring**	0,4143	0,2687	0,8946
*Clean cage less than once a week **	0,2033	0,02022	0,1626
*Clean cage if new rabbit **	0,5421	0,1631	0,4524
Use cleaning agent	1	0,3618	0,1244
Use disinfectant	0,8679	0,3618	0,7516
Coccidiostat in pellets	0,2246	0,05886	0,1912
Diarrhoea	0,5628	0,6641	0,5651
Distended abdomen	0,123	0,1684	0,7275
Anorexia	0,5526	0,4092	0,7489
*Weight loss **	0,1221	0,04392	0,1872
Reduced growth	0,323	0,06907	0,8171
*Disappointing breeding results **	0,97	0,3318	0,9831
*Significant mortality **	0,8899	0,7969	0,9702
*Coccidiosis confirmed **	0,6462	0,5347	1
*Worm treatment **	0,1771	0,2428	0,956