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Prevalence and risk factors for *Eimeria* spp. infection in rabbits in Dutch animal shelters

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Abstract

To study the prevalence and risk factors of coccidiosis and its causative agent, *Eimeria* spp., in domesticated rabbits in animal shelters, 19 official and 14 private shelters sent in randomly gathered, faecal samples from rabbit cages in their facility. The centrifugation-sedimentation-flotation and McMaster method were used to determine the prevalence and quantities of the eleven *Eimeria spp*. known to infect rabbits. A questionnaire regarding housing conditions and hygiene was used to analyse potential risk factors for *Eimeria*-prevalence. 90% of the official shelters and 93% of the private shelters and 72% and 89% of the samples, respectively, were infected. Clinical signs of diarrhoea, growth retardation, anorexia and/or death were present in 10% and 23% of the sampled cages, respectively. Mixed infections were common in the samples: 75% in official and 79% in private shelters. *E. media* and *E. perforans* were found most frequently in both groups. A multivariate analysis found a correlation between prevalence of *Eimeria* spp. and changing cage accessories (e.g. toilets) between cages (p=0,041), as well as a correlation between pathogenic *Eimeria* spp. (i.e. *E. intestinalis, E. flavescens, E. magna* and *E. irresidua*) and cleaning cages daily (p=0,005) and every other day (p=0,017). Since no correlation was found between prevalence nor OPG of *Eimeria* spp. and clinical signs, no conclusion can be drawn about the risk factors for disease. Thus, despite this study showing that infection with *Eimeria* spp. is common in rabbit shelters, more research is needed for definitive conclusions of the risk factors of developing coccidiosis.

Introduction

Coccidiosis is a common disease in both domesticated and wild rabbits. The disease is caused by protozoal organisms of the species *Eimeria.*[1-3] In rabbits, eleven species have been identified, with pathogenicity differing between the species and mixed infections occurring commonly.[1-4] Two forms of disease can be distinguished, i.e. hepatic and intestinal coccidiosis. The hepatic form is caused by *E. stiedae* whereas the intestinal form is most commonly associated with infections with *E. perforans, E. media, E. magna* and *E. irresidua*, of which the last two tend to be more pathogenic.[1-3] The most pathogenic species, however, are *E. intestinalis* and *E. flavescens*.[4] In older animals, infection is usually subclinical[1, 2], but in younger, weanling rabbits, infection may result in severe clinical disease[1-3]. Symptoms of the intestinal form are mild intermittent to severe, watery diarrhoea, growth retardation, weight loss, dehydration, intussusception, rectal prolapse and even death.[1, 2] These symptoms are caused by the damage to intestinal mucosa, which may also predispose for secondary infections (like *Escherichia Coli*). Symptoms of hepatic coccidiosis are due to obstruction of the gall bladder and consist of ascites, jaundice, anorexia and death.[1, 2] Diagnosis can be confirmed by finding high amounts of non-pathogenic oocysts or smaller amounts of pathogenic oocysts in the faeces or bile.[1, 2, 4] Infections are commonly treated with sulphonamides, toltrazuril or robenidine[1, 2], although the last option does not work against *E.*

stiedae[3]. In addition, during treatment, dry and clean conditions should be maintained to prevent the oocysts of *Eimeria* from surviving and infecting other rabbits. However, removing all oocysts may be difficult, due to high environmental resistance.[1-4]
Studies in foreign countries found prevalences varying greatly. In wild rabbits in Egypt, a prevalence of Eimeria spp. of 70% was found. [5] From multiple studies in rabbit farms in China, prevalences of 26% to 70% were deduced. [6, 7] In Kenya, an even higher prevalence of 85% was seen in farmed rabbits. [8] Lastly, in healthy pet rabbits prevalence of Eimeria spp. was 9.5%, but in pet rabbits with diarrhoea it was 63,6%.[9] Although different prevalences were found, all studies found mixed infections of *Eimeria* spp. to be very common, young rabbits to be infected more often as well as more susceptible to developing disease, and poor hygiene to be a significant factor in spread of the disease.[4-7, 9]
Although the disease is common in rabbits, to date little studies have been performed into the prevalence of *Eimeria* spp. in rabbits in The Netherlands. For this reason, two fellow students at the Faculty of Veterinary Medicine set up and conducted a study for the prevalence of *Eimeria* spp. in rabbits in Dutch pet stores. However, there are various other facilities where rabbits are kept together, e.g. whole salers, rabbit shelters, rabbit fanciers/breeders and children zoos. Similar to pet stores, rabbits at these locations can be at increased risk for *Eimeria* infections and associated disease. Thus, it is important to study prevalence of *Eimeria* in these facilities as well. Particularly in animal shelters, where rabbits are often kept together in larger groups, population composition changes regularly, and housing conditions, including hygiene, can be suboptimal, rabbits may be at increased risk for becoming infected with *Eimeria* spp. The aim of the current study was therefore to determine the prevalence of *Eimeria* spp. in rabbits in Dutch animal shelters and to determine potential risk factors for infection with *Eimeria* spp. and subsequently coccidiosis.

Materials and methods

1. IACUC

Since this study is a non-invasive study (collection of faeces and distributing questionnaires) no IACUC (DEC) approval for this study was necessary.

2. Shelters

In The Netherlands, approximately 115 shelters were present in 2016. Of these 115, about 50 were estimated to keep rabbits. [10] Based on prevalences for coccidiosis in other studies varying from 10% to 85%, a mean prevalence of 48% was calculated, based on literature available in 2016. [5-7, 9] Using this number, the required sample size for this investigation was calculated with the program WinEpi. Based on a prevalence of 48%, a population size of 50 and a confidence interval of 95%, a power analysis demonstrated that 45 shelters needed to participate to provide a reliable estimate of the prevalence in Dutch animal shelters.
The shelters were divided in two groups, i.e. ‘official’ and ‘private’. The division was based on registration in the commercial register, qualifications of the caretakers and use of quarantine and isolation. If these requirements were met, the shelter was placed in the ‘official’ group. If not, it was placed in the ‘private’ group. Both types were treated as different groups regarding the prevalence of *Eimeria* spp, to allow comparison and detection of differences between both groups. Regarding the risk factors for coccidiosis, the two groups were assessed together.
Shelters were approached to participate in this investigation using various routes. Official shelters were approached through advertisement in the Shelter Medicine Newsletter or by Internet search for contact information on their website. The private shelters were searched for online, via social media and search engines, and then contacted.
Data were anonymized and each shelter registering to participate received an ID-number. This way, the outcomes of the faecal samples and questionnaire (see below) per shelter could be connected.

3. Animals and faecal samples

Per shelter, three to six cages were randomly selected by the caretaker to be sampled from. One sample was taken per cage and each sample was bagged separately. If multiple rabbits were housed in one cage, the sample would be a mixed sample of the rabbits housed together. Per cage, the age group (see subsection 'Risk Factors'); presenting clinical signs (e.g. diarrhoea, growth retardation, anorexia and death), if any; and potential pregnancy of the present rabbits were noted. Breed and sex were not taken into consideration, since these factors do not influence the risk for the development of coccidiosis. [6, 7, 11-14] Next, from each cage 5 grams of faeces were collected by the caretaker while wearing gloves, and subsequently put into a provided plastic bag. The bags with the samples were labelled with the ID-number of the shelter, the cage number and notification of diarrhoea (if present), following which the bag was placed into a plastic cup with lid. The cup was then wrapped in a tissue and placed into a sealbag, after which it was send back to the University of Utrecht in an airbag envelope, together with the form with animal information. This is according to regulations on sending infectious matters per mail in The Netherlands.

4. Sample processing

Upon arrival at the Centre of Veterinary Microbiologic Diagnostics, the date of arrival was put on the envelope and it was stored in a refrigerated room (6 °C). Samples arriving on a weekday were investigated the same or next day; samples arriving during the weekend were investigated on Monday. This way, at most two days went by before processing.
Assessment of the samples was done by the centrifugation-sedimentation-flotation technique (CSF) and the McMaster method. CSF can be used for differentiation of the oocysts and McMaster for determining the number of oocysts per gram faeces (OPG).
When insufficient faeces was provided, CSF was performed first and the remains were used for McMaster. This was taken into the calculation of the OPG.

4.1 Centrifugation-sedimentation-flotation method

In order to differentiate and roughly count the number of oocysts of the different *Eimeria* species, the CSF method was used as described by the Centre of Veterinary Microbiologic Diagnostics[15]:

1. 1 gram of faeces was grinded with some water;
2. This suspension was filtered and put into a test-tube;
3. Two test-tubes were centrifuged at 3000 rpm for 2 minutes;
4. The surplus water was removed from the test-tubes and replaced with a sucrose-solution (12,7-13,0 g/L) and this was mixed with the sediment with a vortex;
5. A cover slip was placed on top of the test-tubes;
6. The test tubes were recentrifuged at 3000 rpm for 2 minutes;
7. After this, the cover slip was taken of the test-tube and placed onto a labelled microscope slide;
8. The preparate was looked at with a microscope at 10x and 40x enlargement, following which the sporulated and unsporulated oocysts could be differentiated; and the species could be identified and counted to provide a rough estimate.
9. In case differentiation of the oocysts was difficult, a camera-microscope was used to aid in measuring and evaluating the oocyst structure.

Criteria used to differentiate the oocysts were: presence or absence of residuum, presence or absence of micropyle, size and shape of the oocyst. [16] See appendix 1.

4.2 McMaster Method

The McMaster method was used to provide an exact count of the (different) oocysts present in the sample[15]. The method comprised the following steps:

1. A 50 mL Falcon tube was filled with 42 mL of NaCl-solution;
2. 3 grams of faeces were grinded with this solution;
3. The resulting suspension was filtered and placed back in the Falcon tube;
4. The Falcon tube was swayed multiple times;
5. With a pipet, suspension was transferred from the middle of the falcon tube into chamber 1;
6. The Falcon tube was again swayed multiple times;

7. With a pipet, suspension was transferred from the middle of the Falcon tube into chamber 2;
8. Under the microscope the chambers were looked at using 10x enlargement, following which the number of oocysts present were counted;
9. The OPG was determined as 50x(chamber 1+chamber 2).

5. Questionnaire

After the package with materials for sampling was sent, an online questionnaire was sent to the participating shelters by email. This questionnaire was used to determine the risk factors and useful preventative methods for *Eimeria*-infection and coccidiosis. The questionnaire used in the study in pet shops was redesigned to fit in the situation of animal shelters. The new questionnaire consisted of 43 questions, containing:

- 8 questions about general and current occupation of the shelter and quarantine;
- 3 questions about group composition;
- 6 questions about food and water (regimes);
- 8 questions about the cages;
- 8 questions about hygiene precautions;
- 6 questions about health and occurrence of disease;
- 4 questions about prevention and occurrence of coccidiosis in that particular shelter.

The questionnaire is depicted in Appendix 2.

6. Risk Factors

6.1 Age

Rabbits were assigned to the following age groups:

- Z: suckling rabbits (younger than 8 weeks);
- S: weaned rabbits (2-6 months);
- JV: young adult (6-12 months);
- V: adult rabbits (1-6 years);
- O: old rabbits (6 years and older).

6.2 Group composition

Also taken into consideration were the amount of rabbits housed together (single, pairs or groups) and the dimensions of the cage they were housed in. Additionally, it was noted if the shelter ever changes existing group- or couple-compositions.

6.3 Bedding

The different kinds of bottom coverings used in the cages could be filled in in a free choice field. The frequency of replacing this covering was analysed as well:

- Once a day;
- Every other day;
- Twice a week;
- Once a week;
- Never.

Moreover, the frequency of cleaning of the tools that were used for removing bottom covering was taken in:

- Tools were never cleaned;
- Tools were cleaned after cleaning all the cages;
- Tools were cleaned after cleaning each cage;
- Different tools were used for each cage.

6.4 Cleansing

Cleansing was specified as ‘the removal of visible dirt’. The detergents used for cleansing were put in a free choice field and also the frequency of cleansing the cages was analysed:

- Once a day;
- Every other day;

- Twice a week;
- Once a week;
- Never.

6.5 Disinfection

Disinfection was specified as ‘removal of infectious agents’. The used detergents were again put in a free choice field and frequency of disinfection were put into the analysis the following way:

- Once a day;
- Every other day;
- Twice a week;
- Once a week;
- Never.

6.6 Enrichment

Enrichment like toilets and food- and waterbowls are frequently used in rabbit cages. The frequency of cleaning these toilets, and the frequency of cleaning the bowls were analysed separately with the following possibilities:

- Once a day;
- Every other day;
- Twice a week;
- Once a week;
- Never.

Furthermore, an analysis was done regarding these enrichments to be switched between cages.

7. Statistics

7.1 Descriptives

Data from the questionnaires were imported in commercial statistics software (IBM SPSS Statistics for Windows, Version 25.0. IBM Corporation, Armonk, NY). Data were tested for normality using Kolmogorov-Smirnov tests and Q-Q plots. Continuous variables following a Gaussian distribution are displayed as mean ± standard deviation, unless stated otherwise. Categorical variables are displayed as percentage and the number of observations. The level of significance was set at $p < 0.05$, results with $0.05 < p < 0.10$ were considered a trend.

7.2 Risk factors analysis

A multilevel binary logistic regression was used to assess 1) whether there are correlations between presence of *Eimeria spp.* and the presence of pathogenic *Eimeria spp.* and the proposed risk factors and 2) whether the reported clinical signs correlate to the OPG and the present *Eimeria* spp.. All models were built using a step-down building procedure, with cages as level-1 unit and shelters as level-2 unit, in which relevant factors and interactions were included in the initial model. By using likelihood ratio tests, factors that did not significantly contribute to the model fit were excluded from the final model. Correlations between OPG and the proposed risk factors were assessed using linear mixed models. A step-up building procedure was used with cages as level-1 unit and shelters as level-2 unit. Factors and interaction that contributed significantly to the model fit, as assessed using likelihood ratio tests, were included in the final model. All assumptions for the analyses were met.

Results

1. Descriptives

33 rabbit shelters responded to the survey of which 31 (94%) completed the survey, resulting in 159 samples of rabbit faeces. 19 (57%) of the shelters were professional, whereas 14

(43%) were private shelters. In total, 94 and 65 faecal samples were examined from official and private shelters, respectively. These came from 128 and 121 rabbits, respectively.

1.1 Prevalence, clinical signs and OPG

In 17 of 19 (90%) of the official shelters oocysts of one or multiple *Eimeria* species were found. From the 94 samples, 68 were *Eimeria* positive (72%). In 51 of the 68 (75%) positive samples from official shelters multiple species of *Eimeria* were found or oocyst shedding was too low to be detected with McMaster (table 1). The prevalence of each species is listed in table 2 and drawn in figure 1a. Mostly *E. media, E. perforans* and *E. irresidua* were seen and *E. coecicola, E. vejdovskyi* and *E. stiedai* have not been found.
Despite the high percentages of positive samples, only 10% of the rabbits in the sampled cages presented with the above-mentioned signs that could be indicative for coccidiosis. However, in 100% of the official shelters these clinical signs were noticed over the past year (table 3).
OPG in the official shelters ranged between 0 and 25550, with an average of 1938 and a median of 500.

Above-mentioned findings roughly coincide with 13 of 14 (93%) private shelters that were found positive for *Eimeria* spp. Of 65 samples, 58 were *Eimeria* positive (89%). For the private shelters 46 out of 58 positive samples (79%) had mixed infections (table 1 and figure 1b). In table 2 the prevalences of each *Eimeria* spp. can be found. *E. media, E. perforans* and *E. irresidua* were most prevalent and *E. vejdovskyi* and *E. stiedai* have not been found.
In 23% of the sampled cages rabbits were housed that presented with signs that could be indicative for coccidiosis, but 12 of 14 (86%) private shelters did see these clinical signs during the past year (table 3).
OPG in the private shelters ranged from 0 to 12150. The average was 7558 oocysts per gram and the median was 1000 oocysts per gram.

Taking both groups together, the median OPG was 53.8 (0 – 650.0) and the average OPG was 1440.

	Official shelters (prevalence)	**Private shelters (prevalence)**
Rabbits investigated	128	121
Samples investigated	94	65
Samples *Eimeria*-positive	68 (72%)	58 (89%)
Positive samples with mixed infections	51 (75%)	46 (79%)
- 2 species	17 (33%)	9 (20%)
- 3 species	10 (20%)	12 (26%)
- 4 species	19 (37%)	12 (26%)
- 5 species	5 (10%)	9 (20%)
- 6 species	0 (0%)	3 (6,5%)
- 7 species	0 (0%)	1 (2,2%)

Table 1: prevalence of *Eimeria* spp. and prevalence of co-infections in official and private shelters.

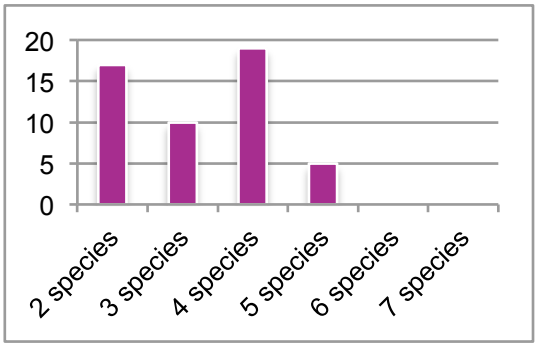

Figure 1a: prevalence of co-infections in official shelters.

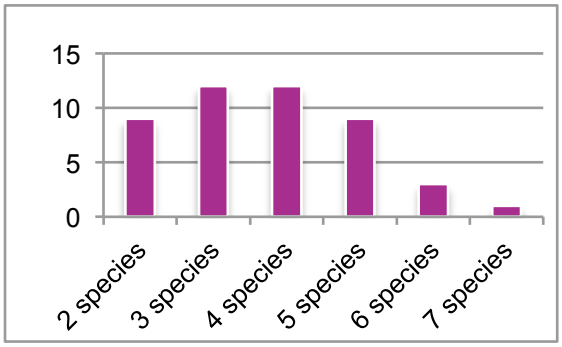

Figure 1b: prevalence of co-infections in private shelters.

Official shelters		Private shelters	
Species	**Samples (prevalence)**	**Species**	**Samples (prevalence)**
E. media	36 (53%)	*E. media*	31 (53%)
E. perforans	34 (50%)	*E. perforans*	31 (53%)
E. irresidua	31 (46%)	*E. irresidua*	28 (48%)
E. magna	14 (21%)	*E. piriformis*	25 (43%)
E. piriformis	5 (7,4%)	*E. magna*	11 (19%)
E. intestinalis	3 (4,4%)	*E. coecicola*	4 (6,9%)
E. flavescens	2 (2,9%)	*E. flavescens*	3 (5,2%)
E. exigua	1 (1,5%)	*E. exigua*	2 (3,4%)
E. coecicola	0 (0%)	*E. intestinalis*	2 (3,4%)
E. vejdovskyi	0 (0%)	*E. vejdovskyi*	0 (0%)
E. stiedai	0 (0%)	*E. stiedai*	0 (0%)

Table 2: prevalences of the different *Eimeria* spp. in official and private shelters.

	Official shelters		Private shelters	
	Shelters (prevalence)	**Rabbits**	**Shelters (prevalence)**	**Rabbits**
Diarrhoea	16 (80%)	227	10 (71%)	248
Growth retardation	6 (30%)	11	7 (50%)	23
Anorexia	15 (75%)	60	7 (50%)	56
Death	17 (85%)	179	10 (71%)	150

Table 3: prevalence of the investigated clinical signs in official and private shelters.

1.2 Risk factors

1.2.1 Age

Age-group was not indicated on 9 samples (5,7%). Weaned rabbits were noted in 23 samples (15%), young adults in 23 samples (15%), adults in 82 samples (52%) and old rabbits in 11 samples (6,9%). Eleven samples came from cages with pairs or groups of different ages ('mixed'; 6,9%). These contained, among others, 4 does with a litter (Figure 2).

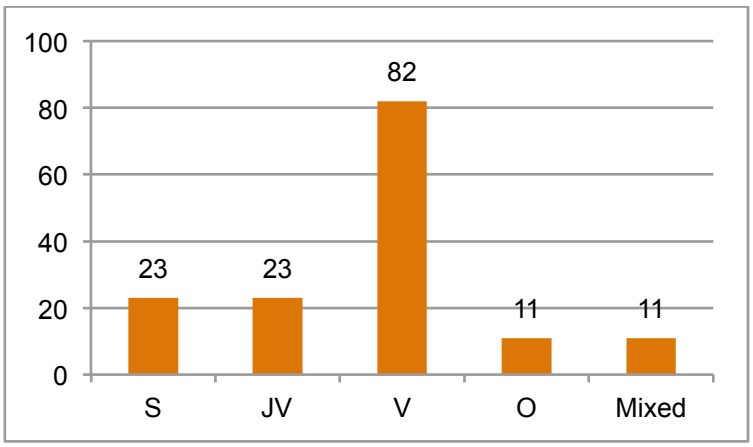

Figure 2: distribution of age-groups. S: weanlings. JV: young adult. V: adult. O: old.

1.2.2 Group composition

The median surface of the rabbit cages was 0.98 m² (0.72 – 4.0 m²), and the cages often contained a single rabbit (107; 67%), followed by rabbits kept as couple (38%) and rabbits

kept in larger groups (15%). However, 17 shelters (52%) indicated that this composition may change over time.

1.2.3 Bedding, cleansing, disinfection and enrichment

The frequency of several cleaning methods of each shelter are presented in Figure 3.
Many different sorts of beddings, cleaning detergents and disinfection detergents were used. These are listed in table 4. Interestingly, only 3 (10%, n = 31) shelters indicated to be steaming the rabbit cages. Unfortunately, because of the great variety in these beddings and detergents, no analysis could be done.
5 shelters indicated to use different tools for changing bedding, 5 indicated to clean them after every cage, 11 cleaned tools after changing bedding of all cages and 10 did not clean the tools at all.

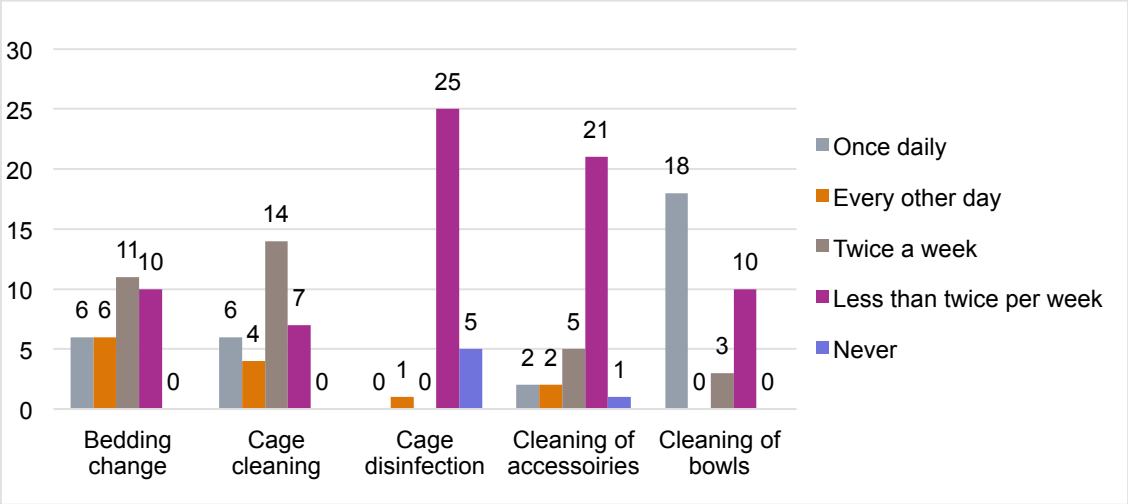

Figure 3: distributions of cleaning- and disinfection-frequencies.

Bedding		Cleaning detergent		Disinfection detergent	
Nature	**Shelters**	**Nature**	**Shelters**	**Nature**	**Shelters**
Straw	29	Water with soap	21	Chlorine	9
Wood fiber	24	Vinegar	6	Halamid	6
Hay	15	Water	3	None	4
Straw-, wood- or paper-pellets	7	Cage cleaning detergent	3	Dettol multipurpose cleaner	4
Flax	5	None	2	Ammonia	3
Hemp	3	Descaler	1	Vinegar	3
Paper	3	Alcohol	1	Steaming	3
Cotton	1			Sabenyl	1
				Cage cleaning detergent	1

Table 4: occurrence of different kinds of bedding, cleaning detergents and disinfection detergents used.

2. Risk factors analysis

Since not all shelters completed the questionnaire, some risk factors were not as well represented as others in the model.
The final multilevel binary logistic regression model consisted of seven variables, of which only the changing of accessories between rabbit cages was significantly correlated to the presence of *Eimeria spp.* in rabbit faeces in the cage (p = 0.041) (Table 5). The likelihood of *Eimeria spp.* infection is higher when accessories are exchanged between rabbit cages when compared to shelters in which this does not occur (OR = 6.096; 95% CI 0.241 – 11.951).
Assessment of the risk factors for infection of pathogenic *Eimeria spp.,* three factors were kept in the final model (Table 6). More frequent cleaning of the rabbit cages lowered the likelihood of pathogenic *Eimeria spp.* infection (p = 0.019), with the lowest risk when cages are cleaned daily (OR = 0.004; 95% CI 0.0007 – 0.188). Interestingly, no correlations could

be found between the presence of clinical signs and OPG (p = 0.145) and the presence of pathogenic *Eimeria spp.* (p = 0.836). No other variables or interactions contributed significantly to the models, and were thus excluded from further analysis.

Parameter	OR	95% CI	p
Frequency of bedding change	-	-	0,346
Frequency of cage cleaning	-	-	0,209
Frequency of cage disinfection	-	-	0,196
Changing cage accessories between cages	6,096	0,241 - 11,951	0,041
Frequency of cleaning cage accessories	-	-	0,344

Table 5: final model on the effect of shelter-related parameters on the presence of *Eimeria* spp. in rabbit faeces in their cages (n=129). OR: Odds ratio. CI: Confidence interval.

Parameter	OR	95% CI	p
Frequency of cage cleaning			
Once daily	0,004	0,0001 - 0,188	0,005
Every other day	0,006	0,0001 - 0,393	0,017
Twice per week	0,674	0,064 - 7,144	0,741
Less than twice per week*			
Frequency of cleaning cage accessories	-	-	0,162
Cage surface	-	-	0,145

Table 6: final model on the effect of shelter-related parameters on the presence of pathogenic *Eimeria* spp., i.e. *E. intestinalis, E. flavescens, E. magna* and *E. irresidua*, in rabbit faeces in their cages (n=136). OR: Odds ratio. CI: Confidence interval. *indicates the reference group for this analysis.

Discussion

An overall remark on this study should be that the calculated sample size was not reached. This makes the study less representative for the population and less reliable.

1. Prevalence

90% and 93% of the official and private shelters were infected and 72% and 89% of the samples from official and private shelters were *Eimeria*-positive, respectively. In the current literature, prevalences range from 9,5% to 94%.[5-9, 11, 13, 14, 17-27] These investigations were done in farmed rabbits, pet rabbits and/or wild rabbits, in regions ranging from the nearby countries of Belgium and Finland, to the continent of Africa and Asia. The studies done in pet rabbits found a prevalence of 94% in rabbits with diarrhoea[21]; 9,5% in healthy rabbits and 63,6% in diarrheic rabbits[9]; 46,2% in pet shop rabbits[11]; 13,51% in rabbits brought into hospital[25]; and 27% in randomly selected rabbit-households.[13] Differences in study design can explain this variety of the 48% calculated in 2016, the prevalences from current literature and prevalences found in this study. Another reason could be the housing of rabbits from different backgrounds in shelters, which could easily introduce *Eimeria* in a naïve population. Plus, these rabbits might have been through stressful events (e.g. moving into the shelter and being coupled with other rabbits), lowering the effectiveness of the immune system. Lastly, in some regions, coccidiostats were used prophylactically[8, 13, 21, 22, 27], contributing to a lower prevalence.

2. Species

In both groups of shelters, *E. media, E. perforans* and *E. irresidua* were the species found mostly. *E. perforans* is found the most common specie in many studies, although it's specific prevalence varies.[6, 14, 17, 19, 21, 28] The regional occurrence and prevalence of *E. media* varies widely, and it is difficult to compare these results of this study to other studies.[6, 7, 11, 14, 17, 19-22,

[24, 28] Yang et al. did not find any *E. media* oocysts at all. [23] In contrast to this study, the literature found mostly low occurences and prevalences of *E. irresidua* [7, 11, 14, 17, 19-22, 24, 28], except for Yang et al. [23] and Yin et al. [6], who found high occurrences.
E. *vejdovskyi* and *E. stiedai* were not found in any sample in both groups. *E. coecicola* was not found in samples from the official shelters and in 6,9% of samples from the private shelters. In only two other studies *E. vejdovskyi* was found, with low prevalences; this roughly coincides with the current study. [20, 24] *E. stiedai* occurred more often than in this study, but also with fairly low prevalences. [6, 7, 17, 20-23] Just Bachene et al. [24] and Hobbs et al. [28] found high prevalences of *E. stiedai*. *E. coecicola* was mostly found around the same prevalence as in this study[6, 7, 11, 14, 17, 19, 20, 22, 23, 28], not at all[21] or in high prevalence.[24]
The species considered most pathogenic, *E. flavescens* and *E. intestinalis*[4], were found in 2,9%-5,2% and 3,4%-4,4% of the samples, respectively. In other studies *E. flavescens* is commonly found with higher prevalences[6, 14, 17, 19, 21, 23, 24, 28], although some studies agree with the findings of this study. [7, 20, 21] Maziz-bettahar et al. and Li et al. did not find *E. flavescens* at all. [11, 22] *E. intestinalis* was overall found in higher prevalences. [6, 7, 14, 17, 20, 21, 23, 24, 28] Razavi et al. and Li et al. did not find any *E. intestinalis* oocysts at all[11, 19]; Peeters et al. and Maziz-Bettahar et al. found prevalences coinciding with this study.[21, 22]
The varying results in the current literature make comparison with findings from this study hard. First, subtle interspecies differences may have caused variety during specie differentiation. Second, unsporulated oocysts could not be identified in this study, possibly causing missing species or divergent prevalences. Third, sampling was done by the caretaker of the shelter. Although a clear description was given of the sampling proces, some mistakes may have been made (e.g., providing dry conditions for the samples, while oocysts do not thrive well in dry conditions[2]; and sometimes not enough faeces was provided). This may have influenced the OPG and the species found.

3. Mixed infections

Studies that checked their data for mixed infections of *Eimeria* spp, found that most samples contained multiple *Eimeria* species[5-7, 17, 19, 22, 23], except for Li et al. [11] The distribution of amount of species in the samples of the private shelters roughly coincides with distributions from the literature. [6, 7, 22] The distribution in samples form the official shelters, however, is solely in accordance with literature regarding the infections with four and five species. [6, 7, 22]
The difference in division of double and triple infections can, again, be due to inability to differentiate unsporulated oocysts, variety of species differentiation or possible mistakes during sampling.

4. Clinical signs

In 10% of the official shelters and 23% of the private shelters, rabbits were showing diarrhoea, anorexia, growth retardation and/or death at the moment of sampling. 100% and 86% of the shelters, respectively, had seen these clinical signs over the past year. Despite the fact that the above-mentioned clinical signs are indicative for coccidiosis, they are not very specific and many other diseases can cause them. Second, observing of clinical signs was done by the caretakers of the shelters and not by a qualified veterinarian. These reasons may have caused the lack of correlation between the clinical signs and OPG, and between clinical signs and presence of *Eimeria*. Also, clinical signs did not correlate with the presence pathogenic species.
The current literature is in disagreement regarding clinical signs. The percentages of rabbits with clinical signs from table 3, coincide with the studies of Okumu et al. [8, 27], but not with the study of Mäkitaipalea et al, who found lower percentages of clinical signs. [13] Interestingly, Mäkitaipalea et al. found that the symptoms of diarrhoea, abdominal pain and anorexia correlated with a lower prevalence of *Eimeria*. [13] Peeters et al. investigated samples from rabbits with diarrhoea and found prevalences of *Eimeria* spp. of 34-47%[21], Laha et al. found a prevalence of 57,28% in rabbits suspected of coccidiosis[18] and Ladron de Guevara et al found a prevalence of 48,3% in diarrheic rabbits[20]; these are not very high prevalences compared to the prevalences in other literature and the current study in mostly healthy animals. However, Lim et al. did find a prevalence of 9,5% in healthy rabbits and 63,6% in rabbits with diarrhoea.[9] Unfortunately, no statistical analyses were done on this data.
Bachene et al. found a prevalence of 47,6% in visibly healthy rabbits, which is in the same

range as the prevalences found in rabbits with diarrhoea.[24] The disagreement in the literature makes comparison very difficult.

5. OPG

Considering the literature, yearly OPG distributions of 150-50000 with an average of 5928 in Indian farms[18], and distributions of 25-142500 with average of 4212 and median of 263 in Finnish pet rabbits[13] have been found. Despite the fact that ranges of OPG are higher in these investigations than in the current study, their averages and median are located in the lower part of the range. This coincides with the distribution found in the current study.
Other studies have found averages in OPG of 859-6950 over one year in different farms in Mexico[20], an average of 42255 from different farms in China[7], averages from 800 to 1500000 in rabbit farms in Algeria[24], averages between 4800 and 63400 in different farms in Southwest China[6] and an OPG-range from 100 to 60000 in Kenyan rabbit farms[27]. The lower ranges of these averages coincide with the average OPG found in the shelters, but the higher ranges surpass the averages of the shelters greatly. This difference could be due to the fact that the majority of the sampled rabbits in the shelters was adult (1-6 years) and rabbits in farms usually are younglings and does.[6, 7, 18, 22-24]From 25 days onward[29], young rabbits tend to be more susceptible to infection and high OPG. [6-8, 11-13, 23, 24] The highest investigated cut-off value for age with a significantly higher OPG was 3 months. [6, 7, 23, 24] Considering this, the most susceptible period to high OPG may lay between 25 days and 3 months. Since 51,6% of the rabbits sampled in this investigation were 1-6 years old, the lower OPG found in this study may be contributed to this. Also in this instance, mistakes in sampling done by caretakers might have contributed to the lower OPG found.

6. Age

Many studies describe a significant difference between age groups for OPG and prevalence. [6-8, 11-13, 23, 24]The highest cut-off value for age with a higher prevalence of *Eimeria* spp. was 6 months. [13] Thus, one would expect the Z-group (1-8 weeks) and/or S-group (2-6 months) of this study to have a significant higher prevalence of *Eimeria* spp., but this was not the case. The lack of correlation between age and prevalence can again be explained by the fact that the majority of rabbits in this investigation was 1 to 6 years old. Furthermore, the age group of 0 to 2 months, had to be put into the 'mixed'-age group, since suckling rabbits are housed with a doe. Thus, this age group, which is part of the susceptible period, was not well represented in the analysis.
However, Ola-Fadunsin et al. [26] also found no correlation and Lim et al. [9] found diarrheic rabbits older than 3 months to have a higher prevalence than diarrheic rabbits younger than 3 months (although no statistical analysis was done).

7. Group composition

The majority of the rabbits was kept alone in a cage, then pairs and then groups. 57% of the shelters changed group composition over time and the remaining 43% kept their rabbits in the same cage and did not move them or add new rabbits. One would have expected that changing group composition would promote the distribution and thus prevalence of *Eimeria* spp., but no correlation was found. Furthermore, no correlation was found between presence of *Eimeria* spp. and group composition (single, pairs or groups). Okumu et al. found higher OPG in rabbits housed in groups compared to single housed rabbits. [8, 27] Furthermore, Mäkitaipalea et al. also found groups of more than two rabbits to be a risk factor for the prevalence of *Eimeria* spp. [13] These differences can be caused by the combination of most rabbits being housed alone (67%) and almost half of the shelters keeping them that way (43%), preventing contamination of other cages with *Eimeria*.
A trend towards significance was found regarding cage surface and the presence of pathogenic *Eimeria* spp. One can imagine that in a smaller cage, the chance of uptake of a pathogenic specie of *Eimeria* will rise. Since less oocysts of the pathogenic species are necessary to cause infection[4] and thus shedding of *Eimeria*, one can explain why only the pathogenic species were correlated to cage surface, and not all species. This can also explain why Okumu et al. and Hobbs et al. did not find correlation between population density and OPG.[8, 12]

8. Bedding

No analysis could be done for the different kinds of bedding used by the shelters in this study, because there was too much variation and too many combinations were being used. In the future, standardisation of bottom coverings should be applied to make proper analysis possible. Okumu et al. did not find any significant difference in types of floors[8, 27], but Ola-Fadunsin found rabbits housed in deep litter to be at higher risk of infection than rabbits housed in tiered cages[26]. A trend towards significance was found for the frequency of bedding change and the prevalence of any *Eimeria* spp. Frequently changing the bedding can prevent the build-up of faeces and thus oocysts, lowering the risk of infection with *Eimeria* spp. [4] This can be supported by the higher risk of infection with rabbits in deep litter. [26] No correlation was found between tool cleansing and prevalence of *Eimeria* spp. The tools used to change the bedding might not provide the conditions oocysts need to survive and thus spread to other cages. No data on this comparison is available in the current literature.

9. Cleaning

Again, due to the high variety in cleaning detergents that were used by the shelters, no correlation could be drawn on this. Standardisation is needed in the future. A trend towards significance was found for the frequency of cleaning and prevalence of all species of *Eimeria*. Regarding the pathogenic species of *Eimeria*, a correlation was found with cleaning the cages once daily and every other day (with daily cleaning giving a lower risk); and a trend towards significance for cleaning cages twice per week. Okumu did not find a significant difference in OPG when comparing housing hygiene[27] or cage sanitation. [8] However, Maziz-Bettahar et al. did find a correlation between high OPG and poor hygiene. [22] Mäkitaipalea et al. did not find a correlation between infection and cleaning the cage once per week or seldom, but did not investigate any other frequencies of cage cleaning. [13] In this study, no significance was found between presence of *Eimeria* spp and cleaning the cage once per week or less, either.

10. Disinfection

The different kinds of disinfectants varied too much for proper analysis and should be standardized in future experiments. A trend towards significance was found between presence of all *Eimeria* spp. and the frequency of disinfection. No other literature is available about cage disinfection in relation to *Eimeria* spp.

11. Enrichment

Cleansing of food- and waterbowls was mostly done every day. No correlation was found between presence of *Eimeria* spp. and the frequency of cleaning these bowls or changing these bowls between cages. Only Okumu et al. investigated the cleanliness of food- and water provisions, as part of cage sanitation, and did not find any correlation with OPG either.[8] However, a trend towards significance was found for the frequency of cleaning cage accessories (e.g. toilets) and the presence of all species of *Eimeria* spp. ánd for specifically the pathogenic species. Mäkitaipalea et al. found no significance between presence of *Eimeria* spp. and cleaning the cage and litterbox once per week or seldom. [13] However, they did not investigate other frequencies of cleaning. A significant difference was found for changing accessories between cages and the presence of all *Eimeria* species. The risk of infection is six times higher when changing accessories compared to not changing accessories. This was to be expected, since accessories, especially toilets, can carry faeces residues and thus oocysts. No other studies have looked at changing of cage accessories.

Conclusion

To the author's knowledge, this is the first study to determine the prevalence and risk factors for *Eimeria* infection in rabbit shelters in The Netherlands.

A prevalence of 72% for official shelters and 89% for private shelters was found. No correlation was found between clinical signs and prevalence of *Eimeria* spp. nor OPG, so unfortunately no conclusions can be drawn about the risk factors for developing disease.

From the results of this study, it can be concluded that the frequency of cleaning rabbit cages is an important factor in the prevention of infection with pathogenic *Eimeria* spp. (i.e. *E. intestinalis, E. flavescens, E. magna* and *E. irresidua*). Cleaning daily has the preference over

cleaning every other day, but the latter seems to lower the risk of infection as well. Furthermore, changing cage accessories between cages seems to increase the risk of infection with all *Eimeria* spp. by sixfold. It would be best not to change these accessories between cages. However, because of the high turnover of rabbits in shelters, accessories will have to be given to new rabbits. A trend towards significance was found for cleaning of accessories and both pathogenic and all *Eimeria* spp., so thorough cleaning can be a solution for this problem. However, since no analysis could be done on detergents, no information can be given on how to clean. Future experiments for risk factors of *Eimeria* infection should be conducted in an experimental setting, with standardized bedding, detergents and frequencies of cleaning.

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Appendix

1. Species differentiation

The information provided by the book *'Biotechnology Guidelines on Techniques in Coccidiosis Research'* made by the European Cooperation in the Field of Scientific and Technical Research, was used for differentiation of the different *Eimeria* spp. of rabbits.[16]

3.1.1.5. Diagnosis from *Eimeria* species from rabbits

From the rabbit more than 25 *Eimeria* species have been described [2] but only 11 species were isolated in pure culture and are well characterized [2, 6]. Among these species *E. media* and *E. coecicola* are difficult to identify when in a mixture with other species. Possibly, the name *E. media* covers several species [2].

Key 5: Key for identification of sporulated *Eimeria* oocysts from rabbits (see also Fig. 15)

Abbreviations: see Key 1.

A. Oocysts without or with inconspicuous micropyle (Fig. 15, A)

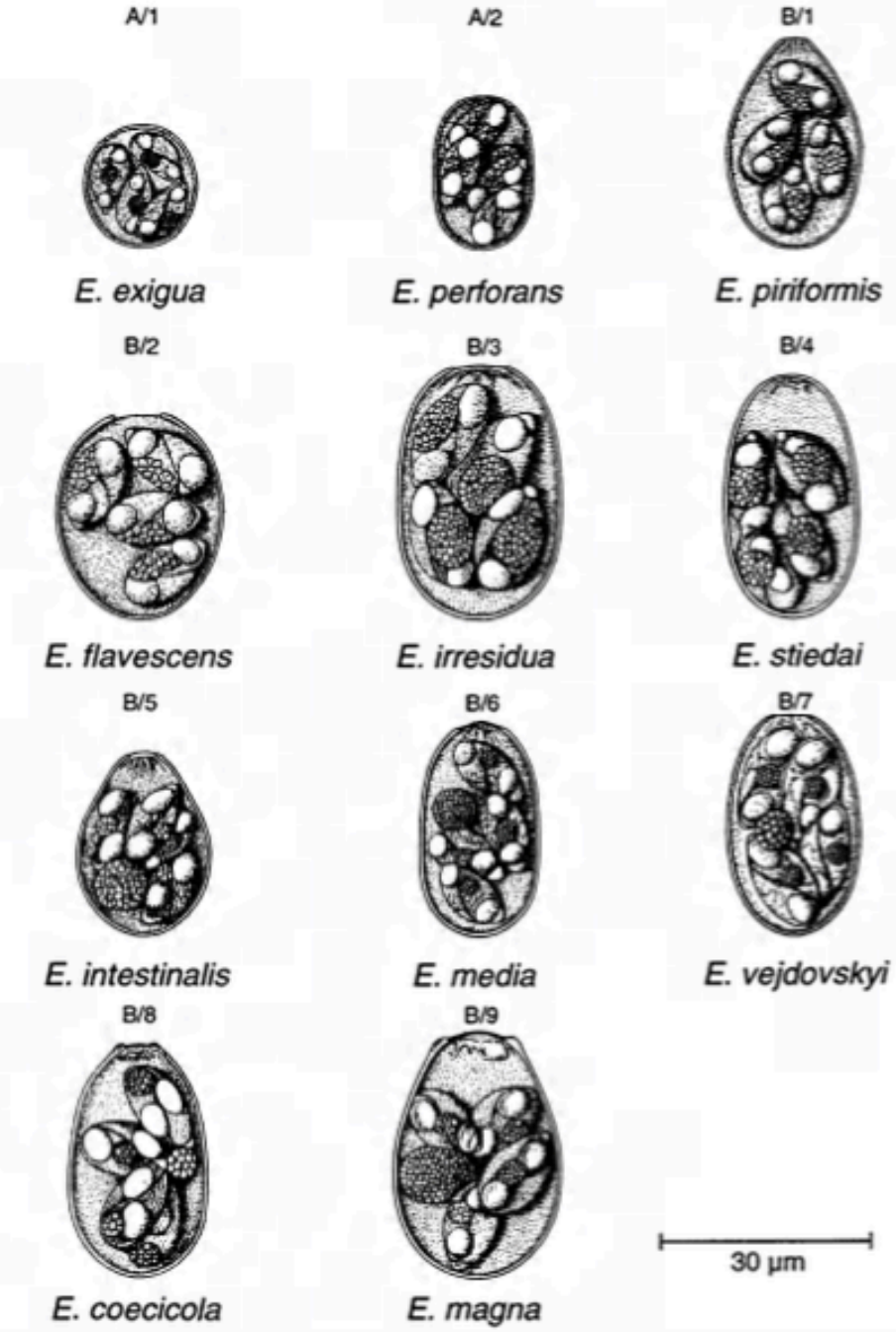
- oocyst spherical or subspherical, colourless, with SR without OR, 10-18 x 11-16 µm (15.1x14.0 µm), ST: 1 d. — ***E. exigua*** (Fig. 15, A/1)

  Note: maybe confused with small *E. perforans*

- oocyst ellipsoid to sub-rectangular, smooth and uniformly thin wall, micropyle very difficult to detect, with SR and small OR, 15-27 x 11-17 µm (22.2x13.9 µm), ST: 1.5 - 2 d. — ***E. perforans*** (Fig. 15, A/2)

  Note: large and medium *E. perforans* maybe confused with small and medium *E. media*.

Fig. 15: ***Eimeria*** species from rabbit



B. Oocysts with micropyle (Fig. 15, B)

Oocysts without oocyst residuum

- oocyst piriform, often asymmetrical, yellowish-brown, prominent micropyle, with SR, 25-33 x 16-21 µm (29.5x18.1 µm), ST: 4 d. — ***E. piriformis*** (Fig. 15, B/1)

  Note: *E. piriformis* is similar to *E. intestinalis* but has no OR.

- oocyst ovoid, yellowish, very large micropyle at broad end, with SR, 25-35 x 18-24 µm (30.0 x 21.0 µm), ST: 4 d. — ***E. flavescens*** (Fig. 15, B/2)

  Note: maybe confused with *E. irresidua*.

- ovoid or barrel-shaped or subrectangular yellowish, micropyle wide, with SR, 31-44 x 20-27 µm (39.2x23.1 µm), ST: 4 d. — ***E. irresidua*** (Fig. 15, B/3)

  Note: maybe confused with *E. stiedai* (micropyle in this species inapparent) or *E. flavescens* (this has micropyle at broad end!).

- oocyst slightly ellipsoid, micropyle almost inapparent, no OR but only a few granules, with SR, 30-41 x 15-24 µm (36.9x19.9 µm), ST: 2-3 d. — ***E. stiedai*** (Fig. 15, B/4)

  Note: maybe confused with *E. irresidua* (but this has distinct micropyle) or *E. coecicola* (is more ellipsoid and has OR).

Oocysts with oocyst residuum

- oocyst piriform, yellowish-brown, large OR (in contrast to *E. piriformis*), with SR, 22-30 x 16-21 µm (26.7x18.9 µm), ST: 3 d. — ***E. intestinalis*** (Fig. 15, B/5)

  Note: see *E. piriformis*.

- oocyst ellipsoid or ovoid, light pink, with medium to large OR and large SR, micropyle with a pyramidal-shaped protuberance. 25-35 x 15-20 µm (31.1x17.0 µm), ST: 2 d. — ***E. media*** (Fig. 15, B/6)

  Note: see *E. perforans*, *E. coecicola*, *E. vejdovskyi*.

- oocyst elongate or ovoid, with SR and medium size OR, micropyle present without collarlike protrusion, 25-38 x 16-22 µm (31.5x19.1), ST: 2 d. — ***E. vejdovskyi*** (Fig. 15, B/7)

  Note: This species may be confused with *E. media* (has a micropyle with pyramidal-shaped protuberance) or *E. coecicola* (has collarlike protrusion around micropyle).

- oocyst elongate-ovoid, with OR relatively smaller than in *E. media*, micropyle with a slight collarlike protrusion and SR, 27-40 x 15-22 µm (34.5x19.7 µm), ST: 4 d. — ***E. coecicola*** (Fig. 15, B/8)

  Note: Small *E. coecicola* maybe confused with medium and large *E. media* and with *E. vejdovskyi* (has no collarlike protrusion).

- oocyst ellipsoid ovoid, dark yellow, truncated at micropylar end with marked collarlike thickening around micropyle, with very large OR and with SR, 31-42 x 20-28 µm (36.3x24.1 µm), ST: 2-3 d. — ***E. magna*** (Fig. 15, B/9)

  Note: sometimes *E. magna* may have a more cylindrical shape and can be confused with *E. coecicola;* in *E. magna* the OR is much larger.

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2. Questionnaire

Enquête i.v.m. onderzoek naar de mate waarin coccidiose bij konijnen in dierenasielen/opvangcentra voorkomt

Hartelijk dank voor uw deelname aan dit onderzoek. Deze enquête bevat enkele vragen over het management en de huisvesting van de konijnen binnen uw opvangcentrum/asiel. De enquête bestaat uit 43 vragen en duurt ongeveer 30 minuten om in te vullen.
Allereerst vragen we u het ID-nummer van uw opvangcentrum/asiel (zoals vermeld in de informatiebrief) in te vullen, zodat wij de resultaten van de ontlastingmonsters kunnen koppelen aan de door u gegeven antwoorden.

1. Wat is uw ID-nummer?

Algemeen

2. Hoeveel konijnen komen er gemiddeld wekelijks binnen?
   - ○ < 1
   - ○ 1-5
   - ○ 6-10
   - ○ > 10

3. Zet u nieuw binnengekomen konijnen eerst in quarantaine?
   - ○ Ja, altijd
   - ○ Ja, maar alleen als de konijnen ziekteverschijnselen vertonen
   - ○ Nee, nooit
   - ○ Weet ik niet / onbekend

4. Hoe zet u nieuw binnengekomen konijnen in quarantaine?

5. Hoe lang zet u nieuw binnengekomen konijnen in quarantaine?
- < 1 week
- 1 week
- 2 weken
- 2-4 weken
- > 4 weken

6. Hoe lang blijven konijnen gemiddeld aanwezig in het opvangcentrum/asiel?
- < 2 weken
- 2-4 weken
- 1-2 maanden
- 2-4 maanden
- > 4 maanden
- Permanent

7. Hoeveel konijnen worden er gemiddeld per week geadopteerd?
- < 1
- 1-5
- 6-10
- > 10

8. Hoeveel konijnen zijn er over het algemeen gemiddeld aanwezig in het opvangcentrum/asiel?
- 1-3
- 4-6
- 7-9
- 10-12
- 13-15
- > 15

9. Hoeveel dieren van elke leeftijdsgroep zijn er in de opvang aanwezig?

Zoogleeftijd (0-6 weken)	
Speenleeftijd (1,5-6 maanden)	
Jong volwassen (6-24 maanden)	

Volwassen (2-6 jaar)

Oud (> 6 jaar)

Groepssamenstelling

10. Hoe zijn de konijnen gehuisvest?
    - ○ Individueel
    - ○ In koppeltjes (2 dieren)
    - ○ In groepen van 3-4 dieren
    - ○ In groepen van 5-10 dieren
    - ○ In groepen van > 10 dieren
    - ○ Alle konijnen zijn gehuisvest in één groot verblijf
    - ○ Anders, namelijk

11. Welk criterium wordt gehandhaafd bij het vormen van groepen?
    - ○ Leeftijd
    - ○ Geslacht

      Toelichting op geslacht:
    - ○ Gedrag jegens elkaar
    - ○ Willekeurig
    - ○ Anders, namelijk

12. Vinden tussentijds veranderingen plaats aan eenmaal gevormde groepen?
    - ○ Ja, er worden nieuwe dieren in reeds gevormde groepen geplaatst
    - ○ Ja, wanneer een konijn alleen overblijft wordt deze bij een ander konijn geplaatst
    - ○ Nee, de groepssamenstelling blijft ongewijzigd totdat de dieren geadopteerd zijn
    - ○ Anders, namelijk

Voeding en drinkwater

13. Hoe wordt het dieet van de konijnen vastgesteld?
    - ○ Alle konijnen krijgen hetzelfde dieet

      Dit dieet bestaat uit (zo specifiek mogelijk)

- ○ Het dieet wordt zo ver mogelijk aangepast aan dat bij de vorige eigenaar
- ○ Anders, namelijk

14. Hoe wordt het voer verstrekt? (Meerdere antwoorden mogelijk)
- ☐ Voerbak
- ☐ Los op de grond van het verblijf
- ☐ Via speelgoed
- ☐ Voederruif
- ☐ Anders, namelijk

15. Hoe vaak wordt het voer verstrekt?
- ○ 2 keer per dag
- ○ Elke dag
- ○ Als het voer op is

16. Hoe wordt drinkwater verstrekt? (Meerdere antwoorden mogelijk)
- ☐ Waterbak
- ☐ Drinkfles
- ☐ Anders, namelijk

17. Hoe vaak ververst u het drinkwater?
- ○ 2 keer per dag
- ○ Elke dag
- ○ Om de dag
- ○ Als het water op is

Informatie verblijf

18. Wat is de lengte en breedte van de verblijven waar de konijnen in verblijven (gemiddeld)?

Lengte (cm)

Breedte (cm)

19. Waar zijn de verblijven gelokaliseerd?
- ○ Alle verblijven staan binnen
- ○ Sommige dieren leven in een binnenverblijf en sommige in een buitenverblijf
- ○ De verblijven staan binnen, maar er is uitloop naar buiten
- ○ De verblijven staan buiten, maar er is een schuilplaats

- ○ Alle verblijven staan buiten

20. Welk criterium handhaaft u bij het wel of niet buiten huisvesten van konijnen?
    - ☐ Leeftijd
    - ☐ Geslacht
    - ☐ Aan- of afwezigheid van wintervacht
    - ☐ Beschikbaarheid van verblijven
    - ☐ Willekeurig
    - ☐ Anders, namelijk

21. Wat is de temperatuur in het binnenverblijf?
    - ○ Onder 18-20 graden Celcius
    - ○ 18-20 graden Celcius
    - ○ Boven 18-20 graden Celcius
    - ○ Onbekend / Weet ik niet zeker

22. Wat voor soort bodembedekking wordt gebruikt in de verblijven? (meerdere antwoorden mogelijk)
    - ☐ Houtvezel
    - ☐ Stro
    - ☐ Strokorrels or papierkorrels
    - ☐ Katoenbedekking
    - ☐ Hooi
    - ☐ Beukensnippers
    - ☐ Anders, namelijk

23. Hoe vaak wordt deze bodembedekking ververst?
    - ○ Elke dag
    - ○ Om de dag
    - ○ 2 keer per week
    - ○ 1 keer per week
    - ○ < 1 keer per week
    - ○ Alleen als er een nieuw konijn / nieuwe konijnen in het verblijf komt / komen (leegstaand verblijf)
    - ○ Onbekend / Weet ik niet zeker

24. Gebruikt u voor het verversen van de bodembedekking bij elk verblijf hetzelfde gereedschap?
- ○ Ja, en dit wordt na het verschonen van elk verblijf gereinigd
- ○ Ja, en dit wordt na het verschonen van alle verblijven gereinigd
- ○ Ja, en dit wordt niet gereinigd
- ○ Nee

25. Mogen bezoekers de konijnen aaien en/of oppakken?
- ○ Ja, aaien en oppakken mag altijd, ook zonder toezicht van een medewerker
- ○ Ja, aaien mag altijd, oppakken alleen onder toezicht van een medewerker
- ○ Ja, maar beide alleen onder toezicht van een medewerker
- ○ Nee

Hygiënemaatregelen
In deze categorie vragen worden twee termen gehandhaafd:
- Reinigen: het verwijderen van zichtbaar vuil
- Desinfecteren: het verwijderen van ziektekiemen

26. Welke middelen worden gebruikt om de verblijven te reinigen?
- ○ Water
- ○ Water en zeep
- ○ Onbekend / Weet ik niet zeker
- ○ Anders, namelijk

27. Hoe vaak worden de verblijven gereinigd?
- ○ Elke dag
- ○ Om de dag
- ○ 2 keer per week
- ○ 1 keer per week
- ○ < 1 keer per week
- ○ Alleen als er een nieuw konijn / nieuwe konijnen in het verblijf komt / komen (leegstaand verblijf)
- ○ Er wordt niet gereinigd
- ○ Onbekend / Weet ik niet zeker

28. Welke middelen worden gebruikt om de verblijven te desinfecteren?
- ○ Chloor
- ○ Ammonia
- ○ Halamid
- ○ Virkon-S

- ○ Onbekend / Weet ik niet zeker
- ○ Anders, namelijk

29. Hoe vaak worden de verblijven gedesinfecteerd?
- ○ Elke dag
- ○ Om de dag
- ○ 2 keer per week
- ○ 1 keer per week
- ○ < 1 keer per week
- ○ Alleen als er een nieuw konijn / nieuwe konijnen in het verblijf komt / komen (leegstaand verblijf)
- ○ Er wordt niet gedesinfecteerd
- ○ Onbekend / Weet ik niet zeker

30. Wordt er stoomreiniging toegepast? Zo ja, hoe vaak?
- ○ 1 keer per week
- ○ 1 keer per 2 weken
- ○ 1 keer per maand
- ○ Alleen als er een nieuw konijn / nieuwe konijnen in het verblijf komt / komen (leegstaand verblijf)
- ○ Er wordt geen stoomreiniging toegepast
- ○ Onbekend / Weet ik niet zeker

31. Hoe vaak worden drinkflessen, water- en voerbakjes, etc. gereinigd?
- ○ Bij het verversen van het water / bij het voeren
- ○ Gelijktijdig met het verversen van de bodembedekking
- ○ Gelijktijdig met het desinfecteren van het verblijf
- ○ Alleen als er een nieuw konijn / nieuwe konijnen in het verblijf komt / komen (leegstaand verblijf)
- ○ Nooit
- ○ Onbekend / Weet ik niet zeker
- ○ Anders, namelijk

32. Hoe vaak worden eventueel aanwezige attributen (bv. schuilplaats, toilet, speelgoed) gereinigd?
- ○ Gelijktijdig met het verversen van de bodembedekking
- ○ Gelijktijdig met het desinfecteren van het verblijf

- ○ Alleen als er een nieuw konijn / nieuwe konijnen in het verblijf komt / komen (leegstaand verblijf)
- ○ Nooit
- ○ Onbekend / Weet ik niet zeker
- ○ Anders, namelijk

33. Zijn drinkflessen, water- en voerbakjes, attributen, etc toegewezen aan één bepaald verblijf?
- ○ Ja
- ○ Nee
- ○ Onbekend / Weet ik niet

Gezondheid

34. Worden de konijnen gecontroleerd door een dierenarts? (Meerdere antwoorden mogelijk)
- ☐ Nee, nooit
- ☐ Ja, eenmalig bij binnenkomst
- ☐ Ja, 1 keer per week
- ☐ Ja, 1 keer per maand
- ☐ Ja, als ze ziekteverschijnselen vertonen
- ☐ Onbekend / Weet ik niet zeker
- ☐ Anders, namelijk

35. Worden de konijnen gevaccineerd tegen myxomatose en rabbit haemorrhagic disease (RHD) bij binnenkomst?
- ○ Ja
- ○ Nee
- ○ Onbekend / Weet ik niet zeker

36. Worden er, eventueel naast vaccinatie, nog andere preventieve behandelingen toegepast bij de konijnen? (meerdere antwoorden mogelijk)
- ☐ Nee
- ☐ Ja, met vitaminen (bv. Vitadrops)
- ☐ Ja, met anti-parasiet (bv. van Beaphar)
- ☐ Ja, met ontwormmiddel (bv. van Beaphar)
- ☐ Onbekend / Weet ik niet zeker
- ☐ Ja, anders namelijk

37. Heeft u bij de konijnen in uw opvangcentrum/asiel wel eens 1 van de volgende verschijnselen geconstateerd? Gelieve de tabel in zijn geheel in te vullen. Indien u het niet zeker weet, vult u *onbekend* in.

	Aantal keer per jaar	Aantal dieren per keer	Aangedane dieren samen in één verblijf
Diarree			
Vermagering			
Groeiachterstand (bij jonge konijnen)			
Sterfte			

38. Wat doet u in geval van sterfte?

- ○ Altijd onderzoek door een dierenarts
- ○ Bij één dier niets, bij meerdere sterfgevallen onderzoek door een dierenarts
- ○ Niets
- ○ Onbekend / Weet ik niet zeker

39. Heeft u wel eens eigenaren die binnen enkele weken na adoptie van een konijn terugkomen omdat het konijn gestorven is? Zo ja, om hoeveel dieren gaat dit per jaar (naar schatting)?

- ○ Ja, 1-2 dieren per jaar
- ○ Ja, 3-4 dieren per jaar
- ○ Ja, 5-6 dieren per jaar
- ○ Ja, meer dan 6 dieren per jaar
- ○ Nee
- ○ Onbekend / Weet ik niet zeker

40. Bent u bekend met de ziekte coccidiose?

- ○ Ja
- ○ Nee

41. Is bij konijnen in uw opvangcentrum/asiel wel eens coccidiose geconstateerd? Zo ja, hoe vaak?

- ○ Ja, 1-2 keer per jaar
- ○ Ja, 3-4 keer per jaar
- ○ Ja, 5-6 keer per jaar
- ○ Ja, > 6 keer per jaar
- ○ Nee
- ○ Onbekend / Weet ik niet zeker

42. Hoeveel konijnen zijn er per keer, dat er coccidiose is geconstateerd, ziek?

- ○ (vrijwel) alle dieren op het asiel/opvangcentrum
- ○ (vrijwel) alle dieren in een verblijf
- ○ Enkele dieren per verblijf
- ○ Individueel dier
- ○ Alleen jonge dieren
- ○ Onbekend / Weet ik niet zeker

43. In geval van coccidiose, waarmee adviseert uw dierenarts u om de dieren te behandelen?

- ○ Niet
- ○ Met Baycox (Toltrazuril)
- ○ Met ESB3 (Sulfonamiden)
- ○ Anders, namelijk

OVERIGE OPMERKINGEN