Dutch roe deer (*Capreolus capreolus*), review of cases presented at the Dutch Wildlife Health Centre

Capreolus capreolus
(source:(Reus, 2013))

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Introduction

The European roe deer (*Capreolus capreolus*) is one of the most common wild mammals in the Netherlands (Rijks et al., 2011). Worldwide, not much deer have been more thoroughly studied than roe deer, from many different perspectives such as: behaviour, reproductive physiology and antler production (Borkowski & Ukalska, 2008; Geist, 1998; Linnell, Duncan, & Andersen, 1998), the detailed information about the biology of roe deer can be found in appendix A.

For many years, the population of Dutch roe deer was growing, from 5000 in 1951 until approximately 64.000 in 2008, which was the last published count (*Reewild, duurzaam beheer.*2012; *CBS; centraal bureau voor statistiek.*31 January, 2013; Montizaan & Siebenga, 2010). During hunting season, hunters try to maintain a healthy population by preventing too high densities and remove roe deer of ill-health from the population (*Reewild, duurzaam beheer.*2012; Montizaan & Siebenga, 2010).

The Latin name, *Capreolus*, literally means little goat (Goss, 1983). This is not a strange name as many pathogens known to infect goats and sheep have close relations with those known to infect roe deer and thereby, many disorders are described to be the same in roe deer, goat and sheep. A summary with general information about diseases of roe deer can be found in appendix B.

However, there are few published papers describing pathogens and diseases in Dutch roe deer and before 2008, little was known about their health status. Since 2008, roe deer are frequently submitted to the Dutch Wildlife Health Centre (DWHC) for post-mortem examination, but an overview of these submitted cases and their pathological findings, has not yet been made.

This study provides that overview, consisting of the diseases and causes of death as observed in roe deer presented at the DWHC at the Utrecht University. This study also defines the most frequently observed pathological findings and their temporal and spatial distribution, which does not necessarily represent what is occurring in the natural environment, but it provides an indication of the distribution of diseases in roe deer in the Netherlands. It also explores the most frequently observed etiological findings and their relation to one or more risk factors.

Materials and Methods

Data from cases before 2013

All of the cases of roe deer submitted to the DWHC for postmortem examination from January 2008 to December 2012 (N=317) were used to create a database. The submitted roe deer were either shot or found dead because of ill-health or found dead due to other causes. From all of these cases, the postmortem records, as can be found in the DWHC Canadian database, were scanned for pathological findings. These pathological findings were categorized into two groups; those '*contributing to the death of the animal on that day in that manner*' (COD) and *'other pathological or etiological findings'* (OTHER). All pathological findings as recorded in the reports under the heading *"final diagnosis"*, which was made by a pathologist, were classified as "COD", all pathological findings found elsewhere in the reports, were classified as "OTHER".
The observed pathological findings were described in terms of topography, morphology and etiology (TME) and compiled into an excel database (Microsoft Office Excel 2003), creating a database which not only included the TME, but also specific host information. This specific host information comprised these items: the roe deer's identification number, date of receiving, province of origin, weight, age category, gender, body condition, longitude coordination and latitude coordination. For the analysis, age category and body condition were subdivided, as detailed in the results.

This analysis was performed using the data of cases obtained through general surveillance and from roe deer submitted as complete or largely complete carcasses (N=240). Excluded from analysis were cases that consisted of a single sample obtained from a carcass such as a head, a spleen, some ticks and/or a tube of blood (N=15). Also excluded from analysis were the cases that were submitted as part of a project (N=62), this project systematically examined all roe deer with closed abdomen and thorax that had died from traumatic lesions consistent with collision in the province of Utrecht in 2010, which implies a different submitting criteria than used for the other submitted roe deer. Including these roe deer in the analysis would increase the sample size unequally, which may lead to biased results.

Data from cases of 2013

Since the aim of this study was partly for the temporal exploitation of the data and since the year 2013 is still incomplete, none of the cases of 2013 were used in the analysis. Though, the participation in six roe deer necropsies gave a better understanding of the descriptions used in the reports and gave an insight in the procedure of a roe deer necropsy.

Statistical analysis

Data analysis was done with SPSS 20 (SPSS 20.0, IBM). The statistical methods used were: t-test for the difference in weights between male and female roe deer, ANOVA and Bonferroni's test for differences in mean weight between roe deer of different age categories, and Odds Ratio's for the relations between a certain risk factor and an etiology or a pathological finding. A number of biologically relevant risk factors were considered and the precise criteria, on which it was decided to use certain risk factors, are stated in the results. The Wald test has been used to test the significance of the Odds Ratio's, for which the level of significance was set at $p=0.05$.

Results

The overview of the pathological and their etiological findings, contributing to the death of the animal, can be found in appendix C and the overview of the pathological and their etiological findings which were classified as "OTHER" can be found in appendix D. For a good overview of the pathological findings, these were presented in one of the following organ systems; "Cardiovascular and hematopoietic systems", "Systemic, Skin, Muscle and Bones", "Nervous system and eyes", "Alimentary system and Liver", "Respiratory system", "Endocrine system", "Reproductive system" or "Urinary tract". The subdivision in these specific organ systems gave the author a better overview of all possible pathological findings. A single animal could have pathological findings in multiple organ systems.

In three (3) cases, within all cases diagnosed with some sort of pneumonia (e.g.: bronchopneumonia, pleuropneumonia and interstitial pneumonia) where the etiologic agent was not determined and was labeled as 'Lungworms not specified', this was replaced with *Dictyocaulus eckerti*. These cases had no additional information about the nematodes in the histology and a clear macroscopic description of the nematodes. This description was of white nematodes of several centimeters (1-5) long and a couple of millimeters (1-2) thick and therefore assumed to be *D. eckerti*.

General characteristics of the roe deer in the sample

Before any relations regarding the most frequently observed pathological findings and their temporal and spatial distribution, or regarding a certain risk factor and an etiology or pathological finding, were explored, the general characteristics of the sample were clarified.

Temporal and spatial distribution

The spatial distribution of the submitted roe deer was not homogenous divided over the 12 provinces in the Netherlands. The distribution was only partially consistent with the density pattern of roe deer in the Netherlands (Table 1). Although Drenthe had the highest density, only a small percentage of roe deer was submitted from this province. Gelderland did not have the highest density of all provinces, but most roe deer were submitted from this province. None of the submitted roe deer came from the province of Flevoland, even though the density in this province was not the lowest in the Netherlands. The temporal distribution showed a peak in the number of roe deer submitted from the province of Gelderland in 2009, while no roe deer were submitted from the province of Utrecht or Overijssel in that year. The exploration of the temporal pattern showed a higher total number of roe deer submitted in the year of 2012.

Table 1. Spatial and temporal distribution of submitted and of all roe deer in the Netherlands (*(Montizaan & Siebenga, 2010))

	Submitted roe deer per year							Total roe deer in the Netherlands*		
Province of origin	2008	2009	2010	2011	2012	Total count	Percent	Total count	Percent	Density per km^2 land surface
Gelderland	11	51	23	19	28	132	55.0	13075	19.9	2.63
Utrecht	2		7	5	11	25	10.4	1396	2.1	1.01
Overijssel	1		4	5	9	19	7.9	10854	16.5	3.26
Noord-Brabant	4	3	3	2	7	19	7.9	9895	15.1	2.01
Limburg		1	4	2	3	10	4.2	4111	6.3	1.91
Drenthe			1	2	5	8	3.3	10391	15.8	3.93
Zuid-Holland			2		5	7	2.9	1156	1.8	0.41
Groningen				3	4	7	2.9	4120	6.3	1.77
Noord-Holland	1		4		1	6	2.5	1535	2.3	0.57
Zeeland		1			4	5	2.1	812	1.2	0.45
Friesland			1	1		2	0.8	5883	9.0	1.76
Flevoland						0	0	2360	3.7	1.66
Total	19	56	49	39	77	240		65588		

Gender

Two different categories of gender, being female and male, were equally divided among the submitted roe deer; there were 119 female roe deer and 119 male roe deer. Only two roe deer were submitted in the third category, *"unknown"*.

Body condition

The body condition in the necropsy reports were constituted of a description of amount of musculature and amount of fat reserves. For the analysis, the categories were divided as follows: *Good, Moderate, Moderate-Poor, Poor, Cachectic* and *Unknown*. The descriptions were placed in a category as listed below.

"Good" included all cases mentioning good condition, as well as cases with sufficient musculature and high fat reserves. *"Moderate"* included all cases mentioning either: moderate condition, prominent musculature, or sufficient fat reserves, as well as cases with combination of sufficient or moderate musculature together with low or moderate fat reserves. *"Moderate-Poor"* included all cases mentioning only a low fat reserve, as well as cases with the combination of sufficient or moderate musculature together with minimal, low or no fat reserves. *"Poor"* included all cases mentioning poor condition, poor musculature or skinny. *"Cachectic"* included all cases mentioning only no fat reserves or cachectic. *"Unknown"* included all cases mentioning unknown, as well as cases mentioning moderate or sufficient musculature together with cachectic. Only a small amount of roe deer were categorized as *"Good"* and the amount of roe deer in the categories *"Cachectic"* and *"Moderate"* were equal towards each other (Fig. 1).

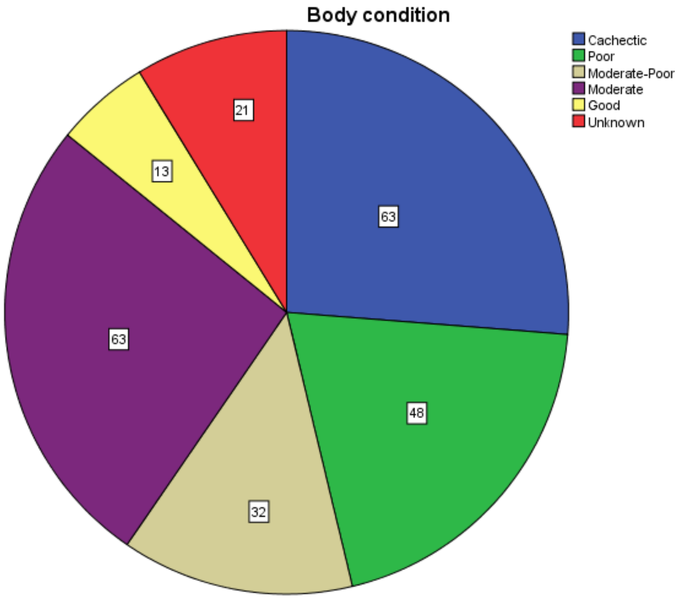

Fig. 1 Amount of roe deer in each body condition category

Age categories

All animals which were classified in the reports as immature (N=39) or neonate (N=1), were categorized as young of the year for the analysis. The different age classes, regarding 'young', 'older' and 'unknown', of the submitted roe deer were close to equal (Fig. 2), about as many young roe deer, consisting of the categories young of the year and juvenile (33%) as older roe deer, consisting of the categories adult and mature (32%) as roe deer with an unknown age (34%) were submitted. However, a greater number of roe deer with an unknown age were submitted in 2012, compared to previous years.

The age categories should be interpreted with some precaution, because establishing the age category has not been done according to a standardized method. The question rose if the subdivision of the roe deer in different age category could be used in analysis. Therefore it is important to know if the subdivision in age categories matches the real age of the roe deer. One way to examine this is to randomly check the subdivision in age categories with the amount of tooth wear, however this was not an option because this was a different and long term project. The decision was made to use the weights of roe deer, where they were present.

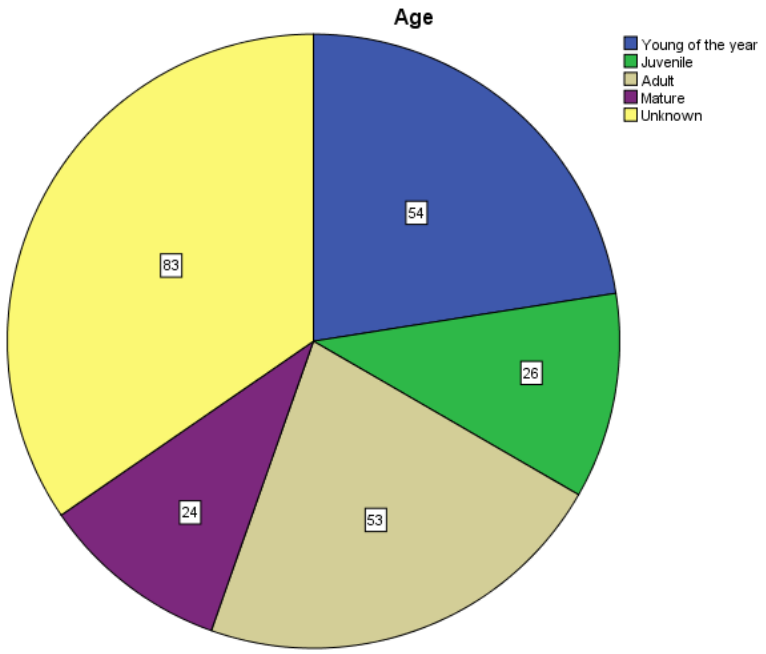

Fig. 2 Amount of roe deer in each age category

Weights

Of all submitted roe deer used in the analysis (N=240), weights were known in 172 cases and these weights were examined for being normally distributed (Fig. 3). Weight can play a role in the differentiation between a young animal and an adult, but a more detailed differentiation would be hard since weight can depend on different factors. In this study, weight was used in order to establish whether the method of subdivision in age category as done in the necropsy room could be used in analysis. A difference in weights between the age categories is expected, as young roe deer are in general smaller than the adults.

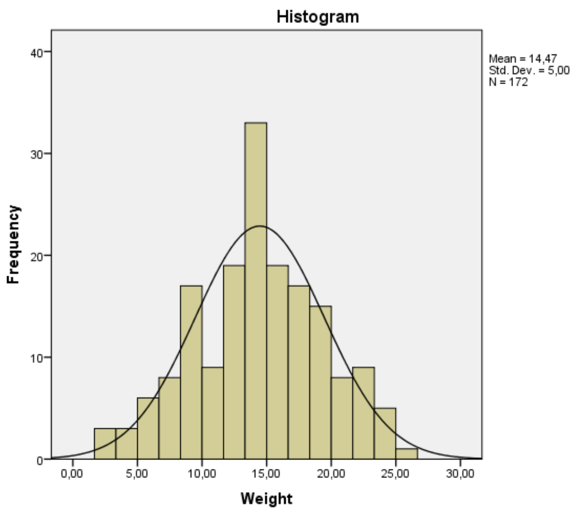

Fig. 3 Distribution of roe deer weights

A significant difference in mean weights between different age categories has been found with an ANOVA (N=172, F=15.1, p=0.000). The subsequent Bonferroni test showed a significant difference in mean weights between the age categories "Young of the year" (11.6 ± 4.6kg) or "Juvenile" (11.0 ± 3.7kg) and "Adult" (16.8 ± 4.1kg) or "Mature"(16.5 ± 4.8kg). There was no significant difference in mean weights between the age categories "Young of the year" and

“Juvenile” and there was no significant difference in mean weights between the age categories “Adult” and “Mature”.
This difference in mean weights was not attributed to gender, since analysis of the data showed no significant difference between female roe deer weight (14.01 ± 4.8kg) and male roe deer weight (14.9 ± 5.2kg; $t(169)=-1.18$, $p=0.239$).

Further exploration of the sample

The most frequently observed pathological finding in the category “COD” was lesions associated with a shot wound (N=116), and the most frequently observed pathological findings in the category “OTHER” were hyperplasia of the white pulp of the spleen (N=147) and the presence of iron (N=112). More detailed information about hyperplasia of the white pulp of the spleen and the presence of iron can be found in appendix B.
The most frequently observed etiologic finding was *Sarcocystis* (N=137) and, as detailed in the section *“Exploration of the most frequently observed etiologies and their relations to certain risk factors”*, no distinction between “COD” and “OTHER” has been made within the etiological findings.
The above mentioned findings are examples of pathologies and etiologies which are described in literature as having little to no clinical relevance in roe deer or in other ruminants (Ammerman et al., 1967; McGavin, 2007; van Ravenswaay, Henry, & Ammerman, 2001; Wall, 2007). Because of this, the above mentioned findings were not accounted for in the exploration.
All of the cases of hyperplasia of the white pulp of the spleen could not be associated with a specific etiology. Further; of all cases of *Sarcocystis*, only one could possibly be related to a pathological finding, being glossitis.

Exploration of the most frequently observed pathological findings in the category “COD”

Numerous pathological findings have been observed which were categorized as “COD” (N=613). The most frequently observed pathological findings contributing to the death of the animal, excluding the trauma due to a shot wound, were: pathological lesions consistent with multiple parasitic infections (N=49) either due to etiologies in multiple organ systems (N=24), alimentary etiologies (N=16), systemic etiologies (N=5) or respiratory etiologies (N=4); pneumonia (N=44); cachexia (N=41) either due to gastro-intestinal etiologies (N=22) or systemic etiologies (N=19); abomasitis (N=21); meningitis (N=12) and anemia (N=7) (Table 2). The relations between these and other pathological findings and their etiologies can be found in appendix C and D. One should notice that most of the cases of pneumonia, cachexia and abomasitis were also caused by an infection with (multiple) parasites. For simplicity, *“pathological lesions consistent with multiple parasitic infections”* will be referred to in text and tables as “Multiple parasitic infection”.

Almost all provinces had animals which had either cachexia and/or pneumonia as pathological finding contributing to their death. On the other hand, Limburg, Drenthe, Zuid-Holland, Zeeland and Friesland did not have any roe deer diagnosed with a multiple parasitic infection, even though this was the most frequently observed pathological finding.

Table 2. Most frequently observed pathological findings in the category "COD" with their spatial distribution

Province of origin	Multiple parasitic infection	Pneumonia	Cachexia	Abomasitis	Meningitis	Anemia	Other	Total submissions per province
Gelderland	37	24	24	15	5	5	238	132
Utrecht	1	4	2	3	1		50	25
Overijssel	4	2	4	1			40	19
Noord-Brabant	5	5	2		1	1	36	19
Limburg		2	1		1		17	10
Drenthe		1	3			1	11	8
Zuid-Holland		2	3				8	7
Groningen	1	1	1				10	7
Noord-Holland	1	1	1	1			14	6
Zeeland		2		1	3		10	5
Friesland					1		5	2
Total	49	44	41	21	12	7	439	

The temporal exploration of the most frequently observed pathological findings in the category "COD" showed some interesting differences in occurrence of pathological findings in 2011 and 2012 compared to other years (Table 3). A smaller number of roe deer have been diagnosed with a multiple parasitic infection in 2012 compared to previous years, while cachexia was less frequently diagnosed in 2011 compared to other years. In absolute numbers, there were more roe deer diagnosed with meningitis in 2012, but the relative frequency was comparable to 2010 and 2011. The numbers of roe deer diagnosed with an abomasitis as a pathological finding contributing to the death of the animal showed a certain consistency over all the years.

Table 3. Most frequently observed pathological findings in the category "COD" with their temporal distribution

Year of submission	Multiple parasitic infection	Pneumonia	Cachexia	Abomasitis	Meningitis	Anemia	Other	Total submission per year
2008	3	1	1	4			38	19
2009	20	8	11	2		1	95	56
2010	10	14	11	5	2	3	78	49
2011	11	10	2	3	3		88	39
2012	5	11	16	7	7	3	140	77
Total	49	44	41	21	12	7	439	240

Exploration of the most frequently observed pathological findings in the category "OTHER"

The spatial distribution of pathological findings which were categorized as "OTHER" was observed to be somewhat equal to the pattern of pathological findings categorized as "COD". The most frequently observed pathological findings in the category "OTHER", with the exception of hyperplasia of the white pulp of the spleen and the presence of iron, were: pneumonia (N=21); enteritis (N=5); abomasitis (N=5); lymphadenitis (N=5); fractures (N=4); meningitis (N=3) and encephalitis (N=3). Most of which were found in Gelderland and none in Noord-Holland, Zeeland or Friesland (Table 4). Meningitis, pneumonia and abomasitis are pathological findings which were observed in both categories. Multiple parasitic infections were only diagnosed twice in "OTHER", in contrast to the multiple parasitic infections in "COD".

Table 4. Most frequently observed pathological findings in the category "OTHER" with their spatial distribution

Province of origin	Pneumonia	Enteritis	Abomasitis	Lymph-adenitis	Fractures	Meningitis	Encephalitis	Total submis-sions per province
Gelderland	12	5	3	1	2	1	1	132
Utrecht	3	1	2				1	25
Overijssel	3							19
Noord-Brabant				1	1			19
Limburg	1					1	1	10
Drenthe				1	1	1		8
Zuid-Holland	1	1		2				7
Groningen	1							7
Total	21	5	5	5	4	3	3	

The temporal exploration of the most frequently observed pathological findings in the category "OTHER", did not share the same pattern as the exploration of those in the category "COD". The latter showed a clear shift for multiple parasitic infections and for cachexia but a comparable shift was not found in the most frequently observed pathological findings in the category "OTHER" (Table 5). However, some deviations have been observed, such as: pneumonia, which has been more frequently diagnosed in 2010 and 2012 and abomasitis, which has been more frequently diagnosed in 2009 and 2010. Lymphadenitis has not been diagnosed before 2011 in "OTHER" or in "COD". It should be taken into account that there were too few numbers of pathological findings in the category "OTHER" in order to obtain relevant results of the temporal or spatial distributions.

Table 5. Most frequently observed pathological findings in the category "OTHER" with their temporal distribution

Year of submissions	Pneumonia	Enteritis	Abomasitis	Lymph-adenitis	Fractures	Meningitis	Encephalitis	Total submis-sions per year
2008	2				2		1	19
2009	3	1	2		1	1		56
2010	7	2	2			1	1	49
2011	2	1	1	3	1			39
2012	7	1		2		1	1	77
Total	21	5	5	5	4	3	3	

The most frequently observed etiological findings and their relations to certain risk factors

In this section, the relations between certain risk factors and an etiology or a pathological finding will be covered. The total number of submitted roe deer which could be used in the analysis was modest and dividing the most frequently observed etiological findings in "COD" and "OTHER" would drastically reduce the sample, thus making the results less reliable.

The most frequently observed etiologies, with the exception of *Sarcocystis* were: *Ixodes ricinus* (N=126), *Dictyocaulus eckerti* (N=93), *Haemonchus contortus* (N=78), *Lipoptena* cervi (N=61), collision or other etiologies consistent with external blunt trauma (N=43), *Cephenemyia stimulator* (N=42) and *Oesophagostomum venulosum* (N=21). In addition, the relation with certain risk factors for the pathological findings "multiple parasitic infections" and "presence of iron" have been explored. A number of risk factors were considered; based upon references in literature or the biologically relevance of a risk, the decision was made for the use of certain risk factors. Different risk factors have been explored for the above mentioned etiological and pathological findings, with the exception of *Ixodes ricinus* and *Lipoptena cervi*, since no relevant risk factors were found in the literature for these pathogens (see also appendix B) (Açici et al., Aug2012; Den Boon et al., 2004; Hodžić, A., Omeragić, J., Alić, A., Jažić, A., Zuko,A., 2012; Kjelland, Ytrehus, Stuen, Skarpaas, & Slettan, 2011; Schouls, Van De Pol, Rijpkema, & Schot, 1999; Wall, 2007; Williams, 2001).

Ixodes ricinus

The main importance of *I. ricinus* is that of the transmission of vector-borne pathogens (Den Boon et al., 2004; Kjelland et al., 2011; Schouls et al., 1999; Wall, 2007; Williams, 2001), but severe infestations with *I. ricinus* could also cause anemia (Wall, 2007). However, diagnosing anemia is not easily done during the necropsy process and in all of the roe deer with diagnosed anemia (N=7), three were associated with *I. ricinus* and/or other bloodsucking parasites, such as *Haemonchus contortus* and *Lipoptena cervi.* Bites of *I. ricinus* can also damage the host, leaving a local site of injury (Wall, 2007). However, of all the roe deer observed with *I. ricinus* (N=126), two were diagnosed with a multifocal alopecia and one with a multifocal dermatitis.

Dictyocaulus eckerti

D. eckerti is a pathogen which affects lungs and especially young animals are susceptible (Chowdhury & Alonso Aguirre, 2001; Wall, 2007). Therefore, the risk factors explored for *D. eckerti* were: age category, being young of the year, juvenile, adult and mature and some sort of pneumonia, being either: bronchitis, (broncho-) pleuropneumonia, (interstitial) bronchopneumonia, (interstitial) pneumonia or pleuritis.
No significant higher odds have been found between any of the age categories and a *D. eckerti* infection. Of all cases of some sort of pneumonia (N=124), 67.7% was caused by *D. eckerti* (N=84).

Haemonchus contortus

Pato et al., 2013 found a higher prevalence of *H. contortus* in young roe deer compared to adult and they also found a higher prevalence in male roe deer compared to female roe deer (Pato et al., 2013). For worms applies: the higher the density of the target animals, roe deer in this case, the higher the risk of infection. So in areas with a high density, a high infection would be expected. Therefore the risk factors explored for *H. contortus* infections were: age, being younger roe deer (consisting of young of the year and juvenile) and older roe deer (consisting of adult and mature); gender and province of origin.
A non-significant higher prevalence for *H. contortus* infection in the older roe deer (32.5%, N=25) compared to the younger roe deer (31.3%, N=25) was found.
Second, a non-significant higher prevalence for *H. contortus* infection in male roe deer (33.6%, N=40) compared to female roe deer (31.9%, N=38) was found and thereby, no significant odds have been found for gender in relation to a *H. contortus* infection.
A significant higher odds have been found for the occurrence of *H. contortus* in Gelderland (N=61), it was 7.3 times higher than the odds of occurrence in Noord-Brabant (N=2). The odds of the occurrence of *H. contortus* in Gelderland was also 20.6 times as high as the odds of the occurrence in Utrecht (N=1). No significant odds have been found between any of the provinces: Noord-Holland (N=1), Overijssel (N=8), Groningen (N=2) nor Drenthe (N=3) compared to *H. contortus* infection. No *H. contortus* infection has been observed in the submitted cases from: Limburg, Zeeland and Zuid-Holland.
H. contortus is a worm that has its predilection place in the abomasum (Wall, 2007). It has been found to be the etiologic agent in 30.8% of the cases of abomasitis (N=26) as observed in the submitted roe deer.

Lipoptena cervi

Infestations with *L. cervi* have been described to induce hyperemia, blood loss and/or an inflammation of the skin (Hodžić, A., Omeragić, J., Alić, A., Jažić, A., Zuko,A., 2012; Wall, 2007). However, of all the roe deer with *L. cervi* (N=61), two were observed with a multifocal alopecia and one with a multifocal dermatitis and all were also associated with an infection with *Ixodes ricinus*. As mentioned before in the section *"Ixodes ricinus"*, diagnosing anemia is not easily done during the necropsy process and in all the roe deer with diagnosed anemia (N=7), only one was caused by *L. cervi*, among other blood sucking parasites such as *Haemonchus contortus* and *Ixodes ricinus.*

Collision

The total sample in this research consisted of roe deer either shot or found dead because of ill-health or found dead because of other causes. The proportion of roe deer that had died from traumatic lesions consistent with collision (for simplicity further named collision) would therefore rather reflect the body condition of the total population of roe deer in the Netherlands. The risk factor explored for collision was body condition, being either cachectic, poor, moderate-poor, moderate and good and the hypothesis explored in this research was that the odds of collision would be higher for roe deer with a better body condition.

The odds of collision were 19.1 times higher for roe deer with a good condition compared to roe deer with cachexia. The odds of collision for roe deer with a moderate condition were 12.2 times higher as the odds of collision for roe deer with cachexia. The odds of collision for roe deer with a moderate condition were 4.4 times higher than for roe deer with a poor condition and 3.9 times higher than for roe deer with a moderate-poor condition.

The odds of collision for roe deer with a good condition were 6.9 times higher than for roe deer with a poor condition and 6.1 times higher than for roe deer with a moderate-poor condition. No other significant higher odds for collision have been found between any of the other body conditions.

Cephenemyia stimulator

Literature describes *C. stimulator* as an obligate parasite that inhabits the throat, pharynx and nasal cavity of cervids. It is not described to cause lesions, but the general effect of *Cephenemyia spp.* is loss of condition (Nilssen, Isomursu, & Oksanen, 2008), and an infestation of throat, pharynx and nasal cavity could also affect lungs. Therefore, the risk factors explored were: body condition, being either: cachectic, poor, moderate-poor or moderate and any form of inflammation in the respiratory system, being either: laryngitis, (interstitial) pneumonia, bronchitis, broncho-pleuropneumonia, (interstitial) bronchopneumonia, pleuritis, fibrosis or pleuro-pneumonia.

No roe deer were observed with *C. stimulator* and a good condition. A significant higher odds was found only once for body condition and the occurrence of *C. stimulator*. The odds of the occurrence of *C. stimulator* for roe deer with a cachectic condition was 10.7 times higher as the odds of occurrence of *C. stimulator* for roe deer with a moderate condition.

No significant higher odds have been found for inflammation in the respiratory system (N=130) and the occurrence of *C. stimulator*.

Oesophagostomum venulosum

O. venulosum follows the life cycle of other trichostrongylids and is therefore only present in spring and summer (Sissay, Uggla, & Waller, 2007; Wall, 2007). A significant higher prevalence and a significant higher risk of infection for *O. venulosum* in male roe deer compared to female roe deer has also been observed in literature (Pato et al., 2013; Segonds-Pichon, Ferté, Gaillard, Lamarque, & Duncan, 2000). Third, a negative relation between body condition and various worms of the digestive tract has been observed (Segonds-Pichon et al., 2000;

Sissay et al., 2007). The risk factors explored for *O. venulosum* were therefore: season, being spring (21-3 until 20-6), summer (21-6 until 20-9), autumn (21-9 until 20-12) and winter (21-12 until 20-3); gender and body condition, being cachectic, poor, moderate-poor and moderate.
The odds of the occurrence of *O. venulosum* in spring are 5.2 times higher as the odds of the occurrence of *O. venulosum* in summer. Of all animals submitted in autumn (N=38) and winter (N=51), none were diagnosed with *O. venulosum*.
No significant odds have been found for the occurrence of *O. venulosum* for male roe deer (N=9) compared to female roe deer (N=12).
No significant odds have been found for the occurrence of *O. venulosum* and any of the body condition categories. However, none of the roe deer with a good condition had an infection with *O. venulosum*.

Multiple parasitic infections

Risk factors which have been explored for multiple parasitic infections were: body condition being cachectic, poor, moderate-poor, moderate and good and province of origin.
The odds of the occurrence of a multiple parasitic infection were 5.1 times as high with a poor condition compared to the odds of the occurrence of a multiple parasitic infection with a moderate condition. Compared to a poor condition, the odds of the occurrence of a multiple parasitic infection were 3.22 times higher with a cachectic condition.
Of all roe deer submitted with multiple parasitic infections a higher odds have been found for the roe deer submitted from the province of Noord-Brabant (8.6 times higher) compared to the roe deer submitted from the province of Utrecht and a higher odds have been found for the roe deer submitted from the province of Gelderland (2.2 times higher) compared to the roe deer submitted from the province of Utrecht. No significant higher odds between any of the other provinces with roe deer diagnosed with multiple parasitic infections (Table 2).

Presence of iron

The presence of iron was found in; spleen, kidney, liver, uterus, lymph nodes and abomasum. Although iron can be found either inside macrophages, other cells or not in a cell, specific notation of where the iron in these roe deer was found was not always clear defined in the reports. Since the different locations of the presence of iron can have different pathological meanings, a more specific notation would be more desirable (McGavin, 2007).
As one can read in appendix B, the two major causes of the presence of iron are: a hemolytic crisis, such as the breakdown of erythrocytes, or ingestion. However, ingestion has to be either; large amounts or small amounts over a long period of time (Ammerman et al., 1967; McGavin, 2007; van Ravenswaay et al., 2001). Therefore, the risk factor explored for the presence of iron was: age category, being young of the year, juvenile, adult or mature.
Only the roe deer in age category juvenile had a significant higher odds (1.2 times higher) on having the presence of iron than young of the year. No significant higher odds have been found between any other age categories and the presence of iron.

Other notable cases and observations

This study was designed in order to explore the most frequently observed diseases and pathogens. Some cases though, were considered to be highly interesting and relevant to discuss briefly.
For instance: two roe deer cases in which Ovine Herpesvirus-2 was established, were incidental cases from the province of Zeeland and could be related to a nearby sheep farm; and *Fasciola hepatica* which was regularly, but not frequently, observed in the submitted roe deer. However, *Fasciola hepatica* is not

very common described in literature to cause disease in roe deer in the Netherlands or across Europe (Chowdhury & Alonso Aguirre, 2001).

Other notable cases were: *Mannheimia haemolytica*, which has been described in literature, (Bojesen et al., 2007), but only found in two pathological findings in one submitted roe deer and *Arcanobacterium pyogenes*, which was found to cause pneumonia in one single roe deer. The general observation was that not much pathological findings were diagnosed with a viral (N=2) or bacterial (N=49) etiology, compared to endoparasitic etiologies (N=302) and that the genus and/or species of the bacteria were only occasionally determined.

Discussion

The aim of this study was to provide an overview of diseases and causes of death observed in roe deer presented at the DWHC in order to define the most frequently observed roe deer diseases with their temporal and spatial distribution. This study also explored the most frequently observed etiological findings and their relation to one or more risk factors.

The spatial distribution of the submitted roe deer was not homogenous divided over the 12 provinces in the Netherlands and was only partially consistent with the density pattern (Table 1). It was remarkable that the highest density was recorded in Drenthe (3.93 roe deer per km^2), but that only a small percentage of the total number of submitted roe deer (3.3%) was obtained from this province. Also remarkable was Flevoland, no roe deer were submitted from this province at all, although the density (1.66 roe deer per km^2) was very similar to that of the province of Friesland, Limburg and Groningen.

The most likely reason for this is that the network might not be optimal developed. The awareness of the existence of the DWHC and thereby the ability to submit diseased roe deer, which has no costs and requires limited effort, might not be present. This can be ascribed to the distance between the DWHC in Utrecht and other provinces. However, a higher submission rate would be expected from Flevoland, since this province is bordered to the province of Utrecht. Only Gelderland and Noord-Brabant, of the five provinces bordering Utrecht, have a submitting percentage of more than five percent. For the provinces of Zuid-Holland and Noord-Holland; this could be due to their different geographical structure which reflects in the density of these provinces. This is not the case for the province of Flevoland, where other factors may influence the submitting percentage.

A second reason for the variation in submitting percentages could be the influence of hunters and rangers. Hunters are allowed to remove diseased roe deer from the population (Montizaan & Siebenga, 2010), but they may feel that there is no reasons to actual submit the animal for further examination. Personal interest may also play a role in the consideration to submit a diseased roe deer as 71 roe deer were submitted by three different persons from two different provinces, being Gelderland and Utrecht. Also, the total roe deer count was done by hunters and rangers, differences in accuracy between hunters or rangers from different provinces could affect the total count and thereby the density.

The third and last reason to discuss is the possibility of a different prevalence of certain diseases among provinces. Even though the occurrence of the abomasal worm *Haemonchus contortus* has been explored for different provinces, no conclusions can be drawn from this exploration since the total numbers of submitted roe deer were too small. Data needed for the exploration of the prevalence of diseases and pathogens in different provinces was only partially available and beyond the subject of this study.

The temporal distribution of the submitted roe deer showed a peak in the number of roe deer submitted from the province of Gelderland in 2009. This might be due to the fact that the DWHC was only recently introduced in this province in 2009; however, other reasons, such as a disease occurring in the province, cannot be excluded. The temporal distributions also showed a higher total number of roe deer submitted in 2012, this might be due to a growing network. However, a longer termed study has to be performed in order to get data that supports this statement.

All conclusions were made and should be read keeping in mind that the sample does not represent the total population since mainly sick and weak roe deer have been submitted for postmortem examination. It is not a random sample, and does not match with roe deer densities in the Netherlands.

The most frequently observed pathological finding contributing to the death of the animal was a multiple parasitic infection. Thereby, one should notice that most of the cases of pneumonia, cachexia and abomasitis had a (multiple) parasitic infection as etiology. A long-termed study in Sweden showed a different distribution in most frequently observed causes of death. Trauma, winter starvation and gastritis/enteritis, closely followed by bacterial or parasitic infections, were the most common causes of death. However, previous studies in Sweden showed remarkable more infectious and parasitic diseases than winter starvation (Aguirre, Brojer, & Morner, 1999). A variety of literature can be found on the topic of parasites being the reason of a decline in wildlife populations (PEDERSEN, JONES, NUNN, & ALTIZER, 2007; Williams, 2001; Woodroffe, 1999), but few literature studies were found on causes of death in roe deer. In the Netherlands; Limburg, Drenthe, Zuid-Holland, Zeeland and Friesland did not have any roe deer diagnosed with a multiple parasitic infection, even though this was the most frequently observed pathological finding. This could be due to a lower submitting percentage from these provinces. However, almost all provinces had roe deer submitted which had either cachexia and/or pneumonia as pathological finding contributing to their death, while the multiple parasitic infection was the most frequently observed pathological findings. Since cachexia was a major reason for submitting roe deer, the conclusion could be made that pneumonia occurs as a nationwide problem.

The temporal exploration of the most frequently observed pathological findings showed some interesting differences. A multiple parasitic infection has been less frequently diagnosed in 2012; meningitis and lymphadenitis on the contrary have been more frequently diagnosed in 2012. These differences might be due to a change in infection rate with certain pathogens, but the greater amount of roe deer submitted in 2012 could also be the main cause. This latter showed in the relative frequencies, which were quite equal to other years for the case of meningitis. Other causes which could have taken part in these differences were: a different manner of sampling or investigating the tissue; or new insights concerning the disease. Cachexia, on the other hand, has been less frequently diagnosed in 2011 compared to other years. A variation in the weather conditions is one of the factors considered to play a role in this as weather conditions influence the growth of vegetation. However, the exploration of this change in weather conditions was beyond the subject of this study. Not much conclusions can be drawn from this temporal exploration, as it only covers a couple of years. A longer termed study is required if one is interested in the temporal distribution of the occurrence of certain diseases.

In some of the explored relations, the observed odds were consistent with what was found in literature or with what was expected in the hypothesis, these were: *Oesophagostomum venulosum*, which was expected to be present in spring and summer (Wall, 2007) and was observed to be significantly higher in spring, none were found in autumn or winter. The second was *Cephenemyia stimulator*, an obligate pathogen of roe deer which does not cause any lesions other than loss of condition (Nilssen et al., 2008; Wall, 2007); it was observed to have a higher odds in animals with a lower body condition. Another example was the multiple parasitic infections, which had higher odds in roe deer with a lower body condition. But, as stated in the recordings of the cases, infections with multiple parasites could be a reason for weight loss; however, weight loss could lead to a decrease in immunity, which can lead to a higher infestation with parasites. Exploring the spatial distribution of roe deer with a cachectic body condition could be a subject for further research as more data is required for a more reliable result. *D. eckerti* has been described to cause severe inflammatory of the respiratory system (Chowdhury & Alonso Aguirre, 2001; Wall, 2007) and 67.7% of all cases of some sort of pneumonia (N=124) were caused by *D. eckerti*. The odds of collision were also consistent with the hypothesis since a higher body condition was associated with higher odds of collision.

On the contrary, other observed odds differ from what was found in literature, such as: *O. venulosum*, in which no significant difference between different body conditions were found nor a difference between male and female roe deer (Pato et al., 2013) and the presence of iron, which was not significant related to age (McGavin, 2007). Another example was the odds of an infection with *H. contortus*, no significant odds were found for age or gender in contrast with what is known from literature (Pato et al., 2013). Besides this, higher odds of a *H. contortus* infection were expected in provinces with higher densities. However, no significant higher odds have been found for Overijssel or Drenthe. The odds of a multiple parasitic infection were also expected to be higher in provinces with higher densities, but no significant higher odds were found for Overijssel and no significant lower odds were found for Noord-Holland. Based upon these results, it cannot be concluded that density plays a role in infection rate. The last difference of odds with what has been described in literature was found in cases with *Dictyocaulus eckerti*, which has been described to be a pathogen which mainly infects young animals (Chowdhury & Alonso Aguirre, 2001; Wall, 2007). However, age was not a significant risk factor for the occurrence of a *D. eckerti* infection in this study.
These differences in what is known from literature and what is found in this research, are believed to be the result of a small and non-randomized sample. However, it is also possible that the situation in Dutch roe deer does differ from what is known in literature, since these studies have not been described for roe deer in the Netherlands. It might also be the result of different criteria for including roe deer in the sample as other researchers might have used a more randomized or a more representative sample. At last, the results were also influenced by the different submitting percentages among provinces.

A difference between mean weights of young roe deer, being young of the year and juvenile; and older roe deer, being adult and mature, has been found. Therefore we conclude that the subdivision between a young and an older roe deer in generally can be used. But a conclusion based upon the results about the subdivision in four age categories cannot be made, since no differences were recorded between the mean weights in the four different age categories and since weight can depend on much more factors than age. In 2012, a greater number of roe deer with an unknown age were submitted even though a protocol has been established, which can be used to estimate the age in the necropsy room.

Many of the smaller gastro-intestinal worms, known to cause diarrhea such as *Nematodirus Europaeus* (Chowdhury & Alonso Aguirre, 2001; Wall, 2007), were seldom diagnosed in this study. Either the prevalence in the Netherlands is low, or they are missed because of their size and methods needed for retrieving. Several researchers have described *N. Europaeus*, which is a roe deer specific *Nematodirus spp.*, as the most common *Nematodirus* species among wild ungulates in the Netherlands (Borgsteede et al., 1990; Dróźdź, Demiaszkiewicz, & Lachowicz, 1992; Jansen, 1992; Pato et al., 2013; Rossi, Eckel, & Ferroglio, 1997; Váquez et al., 01/2010). The method for retrieving the *N. Europaeus* contains the washing of the part of the intestine with the most prevalence for these worms, filtering the contents and examining them under a microscope (Pato et al., 2013; Wall, 2007). Since this last is not done systematically during the necropsy process, *N. Europaeus* and other smaller gastro-intestinal worms are believed to be missed.

To conclude, the general observation was that not much roe deer were diagnosed with a viral (N=2) or bacterial (N=39) etiology, compared to a parasitic etiology. Cases in which a bacterial or a viral etiology is suspected were relatively rare, further diagnostics is highly recommended to obtain more understanding of these etiologies in future roe deer cases. The second observation was that despite the fact that multiple parasitic infections were the most frequently observed pathological finding contributing to the death of a roe deer in the Netherlands and that a different submitting percentages occurred in all provinces, only pneumonia was observed in almost all submitting provinces. Closer monitoring of this disease is recommended in order to find an underlying cause. Third, there seemed to be a pattern in occurrence of certain disease among different years; a lower occurrence of multiple parasitic infections in 2012 and a lower occurrence of cachexia in 2011. It is suggested to conduct a longer-termed study in order to find if these were annual differences or if there might be an underlying cause. Last, there has been an unequal spatial distribution which is most likely caused by a difference in the awareness of the existence of the DWHC among provinces. Recommended is to expand the existing network from provinces with a high submitting rate, in to the provinces with a low submission rate.

Although this research gave an overview of diseases and causes of death in Dutch roe deer and risk factors have been explored for different etiologies, it is expected to raise questions. Some of these could be a subject for further research some might remain unanswered. It is suggested to continue with making overviews as these provide insights in certain patterns. However, these insights become more realistic with a more randomized sample and with a higher number of roe deer.

Appendix A: The biology of *Capreolus capreolus*

The European roe deer (*Capreolus Capreolus*) is one of the most common wild mammals in the Netherlands (Rijks et al., 2011). Worldwide, not much deer have been more thoroughly studied than roe deer, from many different perspectives such as; behaviour, reproductive physiology and antler production (Borkowski & Ukalska, 2008; Geist, 1998; Linnell et al., 1998). The population of roe deer in the Netherlands was estimated at 64.000 in 2008 (Montizaan & Siebenga, 2010).
In Western Europe live, approximately, six million roe deer, making it the most numerous wild *cervid* species in Western Europe (Fickel & Reinsch, 2000).
During the hunting season, which lasts from the 1st of April 2013 until the 15th of September 2013 for bucks and from the 1st of December 2012 until the 16th of March 2013 for fawns and female roe deer, rangers try to maintain a healthy population by preventing too high densities. These periods can vary among different provinces, depending on the granted exemption (*Wildkalender jachtwild.*2012). Given its high population density, the management of Dutch roe deer is not so much orientated at conservation but rather at road safety and the production of venison (Geist, 1998; Montizaan & Siebenga, 2010).

The subfamilies of *Cervidae* can be classified in different ways and the main classification can be based upon: fossil records, geographic distribution, anatomy of the legs and/or the number of chromosomes. Classification based upon the number of chromosomes should be carried out carefully, but correlation to other characteristics makes this more credible.
The classification based upon the anatomy of the legs, as described by Sir Victor Brooke, is based upon the anatomy of the second and fifth metacarpal bones. For this, there can be distinguished between animals which possess only the more distal elements and animals which also possess the more proximal remnants as well. Sir Victor Brooke called them telemetacarpalia and pleisometacarpalia respectively. According to this, roe deer would be telemetacarpalia (Goss, 1983).

Origin and distribution

Three deer came from the high-latitude forests of cold-temperature in North America and Siberia; moose, reindeer and roe deer. All three are telemetacarpalians and probably share the same origin (Geist, 1998). They have begun to spread throughout Europe at very early ages. Depending on the topography of the land and the level of hunting practiced, the population can vary from 1 up to 50 animals per 1000 hectares (Chowdhury & Alonso Aguirre, 2001). In the Netherlands, this density lies between 5 to more than 25 animals per 100 hectares (*Reewild, duurzaam beheer.*2012).

Since when the first roe deer were present in the Netherlands, is not quite clear. The earliest remains date from the late Atlantic period, which was roughly around 6.000 until 4.000 BC, but many differ in opinion about the exact dates. Although, given the recent knowledge, the European roe deer had to spread through Dutch territories in order to reach England. Therefore, they should have been in the Netherlands somewhat equal or slightly earlier than their arrival in England, which was approximately 8.000 BC. It is also stated in historical sources that roe deer, during the late Middle Ages, inhabited the dunes along the coast (Hufthammer & Aaris-Sørensen, 1998).

The European roe deer is widespread in Western Europe and occurs in every European nation, with the exception of the most northern regions of Scandinavia, including the islands. It is also not found on Iceland, Ireland and the islands in the Mediterranean Sea.

Closely described, their habitat ranges from Norway in the north, the Ural Mountains in Russia in the east, the northern regions of Turkey until the Caucasus (including Georgia, Armenia, Azerbaijan and Iran) in the south-east, the Mediterranean Sea in the south, Portugal in the south-west and Finland in the north-west (Figure 4)(Chowdhury & Alonso Aguirre, 2001; Fickel & Reinsch, 2000; Lister, Grubb, & Sumner, 1998). Roe deer habitat ranges from 50° North latitude until 35° North latitude, but other sources state these are 68° and 36° North latitudes (Hartl, Hewison, Apollonio, Kurt, & Wiehler, 1998).

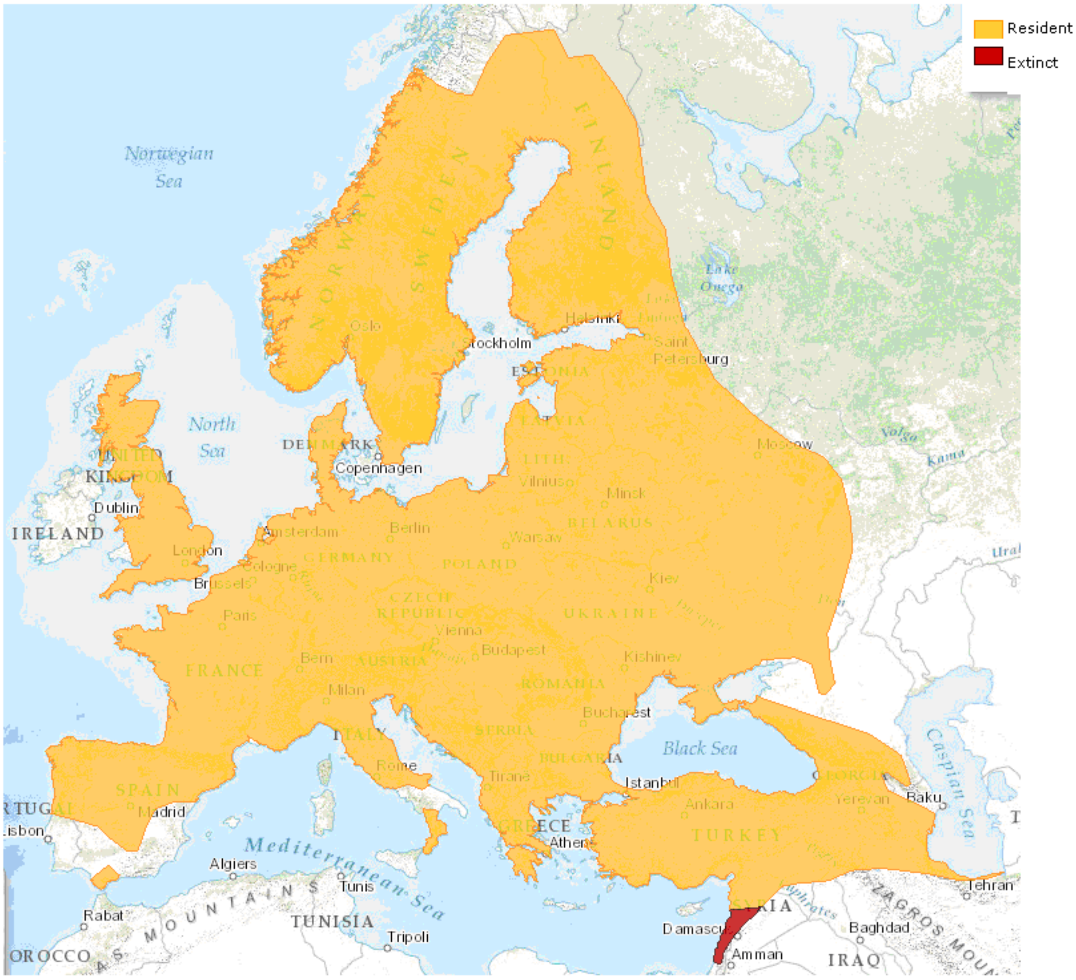

Fig. 4 Distribution of *Capreolus capreolus* in Europe (source: (IUCN Red List of Threatened Species, 2012))

Roe deer live in a wide range of areas with different vegetation, including; decidicuous, coniferous and Mediterranean forests, but also; shrublands, moorlands and marshes (Chowdhury & Alonso Aguirre, 2001; Linnell et al., 1998). Open grasslands and high alpine areas are generally avoided. Roe deer are relatively small animals, which allow them to survive in small patches of woodland or shrubs and even tall grass. Extreme climates are not dangerous to roe deer, as they are extremely tolerant. Which is most highly unexpected, since larger animals, due to their body size, seemed to be better adapted to extreme environments (Sempéré, Mauget, & Mauget, 1998).

External morphology

The European roe deer is a relatively small animal. It has a body length of 95-135 cm, a shoulder height of 65-75cm and a weight of 15-35kg (Macdonald & Barrett, 2001), although the references for length, height and weight can vary a lot throughout literature. Chowdhury et al. stated that roe deer males can weigh up

to 59kg, but the females are usually smaller (Chowdhury & Alonso Aguirre, 2001).
In the southern parts of the Netherlands, measurements have been done both at bones and skeleton of roe deer. Only few remnants were available, therefore neither conclusions nor detailed studies could be made, but the measurements do indicate that roe deer were large animals of comparable size with the Danish deer (Hufthammer & Aaris-Sørensen, 1998).

Roe deer have a facial gland in front of each eye (Frey, Markgraf, & Hofmann, 2001; Goss, 1983). This gland contains a pheromone, which is used to mark the territory of the roe deer. These glands are opened by bucks in case of anger or excitement. They also have metatarsal glands, which are opened when roe deer are alarmed (Geist, 1998). Roe deer do not have a gallbladder, but they do have a tapetum lucidum, which gives them sufficient night vision.

Roe deer have a typical look; they are small with a relatively short and broad head. They have a black band around the muzzle, this band is interrupted by white spots just below the rhinarium and they have a white chin. Roe deer have large ears, a long neck, a short trunk and long limbs, with the hind limbs being longer than the forelimbs.
Their coat has two different appearances; the winter pelage is grey with an agouti-banding pattern. The pale hairs are darkening towards the tips and they have a subterminal paler band. The summer coat, on the contrary, lacks the agouti-banding pattern. The hairs are shorter and thinner and are bright orange-brown. Their short tail is surrounded by a white caudal disc which, during summer, can be less distinct or even absent. The fawns are typically spotted and are distinct from other species such as *Dama Dama, Axis Axis* and *Cervus Elaphus*. These species have two lines of spots which are almost meeting in the middle of the back. The roe deer fawns have a pattern which is similar to odocoileus species and will loose these spots by the end of their first winter (Lister et al., 1998).

Female roe deer raise their fawns during summer, while the bucks defend their mating territories, which is a more or less solitary organization. During late autumn and winter, herds will be formed. These are not fixed but more instable herds and the size of the herd varies with population density and habitat openness, but varies between 5 or 6 to several dozens of deer. The organization within these herds can vary; mostly they consist of one or two female roe deer, usually sisters, and their offspring. Occasionally, one or two male bucks can join these herds (*Reewild, duurzaam beheer.*2012; Chowdhury & Alonso Aguirre, 2001; Kurt, Hartl, & Voelk, 1993; Linnell et al., 1998; van der Wal, 2012).
Distinguishing between forest and field roe deer can be made, but this is not very common in The Netherlands. Forest roe deer have a typical stable structure of the herd with a mother family and one dominant buck. While the field roe deer are more gregarious during winter but have a more unstable herd after crop harvest, due to restructuring. There are also quite a few other differences between the two populations, but these are rather due to the environment influences than due to genetics (Hartl et al., 1998).

Roe deer are saltatorial runners, but they are incapable of running far and fast and are therefore experts in hiding. The former mentioned features; their small body size, color of their coat, low weight and preference for certain types of vegetation, gives them the necessary requirements for hiding (Geist, 1998; Linnell et al., 1998).

Many deer species possesses antlers which are not the same as horns, since the former are branched and composed of solid dead bone. Antlers grow their

hardness by creating successive layers of cornified epidermal cells. Horns are permanent structures, while antlers are shed each year and renewed through apical growth centers. The mechanisms behind the phenomenon of shedding and renewing antlers annually, remain unknown and unresolved. They are open to all kinds of interpretations (Goss, 1983).
The formation of antlers in most deer depends on the rise and fall of testosterone secretion. When the sex hormones are at a minimal level and the fertility low, the antlers grow. The rise in testosterone nearby rutting season induces the antlers to mature. These hormonal fluctuations are caused by changing day length (Goss, 1983).
Antlers are only grown by male roe deer, with the exception of female reindeer as they are the only female deer that grow antlers. The pedicles, from which the antlers originate, are situated at the top of the head. Sometimes, these pedicles can be so close that the antlers are partly fused in the midline. The antlers are pointing almost straight upwards before they branch.

The cycle by which the antlers grow, are unusual compared to other deer. Other deer shed their velvets just before the winter, cast their antlers in spring and immediately start growing a new set during spring and summer, just before the mating season. The roe deer on the contrary, and also the Père David's deer (*Elaphurus davidiensis*), are the only deer to grow their antlers throughout the winter, directly after the casting and they shed their velvets in early spring (Goss, 1983). Père David's deer was formerly inhabited in China, but became completely extinct, nowadays it only lives in various parks throughout Europe and is being reacclimatized (Chowdhury & Alonso Aguirre, 2001).
During their first year of life, around the age of 6 to 8 months, fawns grow a small set of antlers. They grow these antlers from November until February, in February they shed their antlers and they will not regrow their antlers before the next winter, when they are 1.5 years old (Goss, 1983).

The pedicles and primary antlers are the result of the neogenesis of bony structures, whereas the formation of the secondary antlers is a regenerative event. This regenerative event, as occurs in antler formation in deer, is not found in other mammals. The primary antlers are formed on a specialized periosteum, which is located on the distal parts of the cristae externae on the frontal bones. The rising androgen levels in the fawn's blood, causes determined periosteal cells to get activated after which they autonomous start the antlerogenic, the forming of antlers. This specialized periosteum can be transplanted to heterotopic locations, such as a limb, where it will still grow antler structures. The skin plays a passive role in antlerogenic; it reacts to the formation of antler bone by forming the velvet (Brown, 1992).

Reproductive and life cycle

The first time of giving birth is the same for almost all female roe deer and is at the age of two years. After this, they will give birth every year to one, two or sometimes even three fawns in May or June (Sempéré et al., 1998). The mating starts in early fall, the monoestrus females will produce two oocysts, one from the left and one from the right ovarium, thereby often producing a dizygotic twin (Andersen, Gaillard, Linnell, & Duncan, 2000; Huygen & Poutsma, 2000). The first reproduction cycle begins at the age of 16 months.

Ordinary *cervids* start their rutting after the complete renewal of the male antlers, which will be in mid-summer. Hereafter, mating will take place in autumn, from October until December, in order for the females to give birth in spring, which is April to June (Geist, 1998; Sempéré et al., 1998). Almost all cervids are polyestrous and the ovulation depends on the length of the day and is induced by shortening of the photoperiod. This also accounts for the rutting, which is controlled by testosterone and depends on decreasing day length too.

Roe deer are no ordinary *cervids*, as aren't Père David's deer. The latter will rut in late summer and gives birth in early summer, due to its long gestation, which can be up to 9 months (Sempéré et al., 1998). Female roe deer are monoestrous; therefore the actual breeding season may be very short. Although the roe deer bucks have a long period of sexual activity, about 7 months, the female roe deer can only be fertilized during 36 hours. Roe deer are also known to have a delayed implantation, the explanation of the mechanisms behind this are highly speculative (Sempéré et al., 1998). It is described that the ovulation is either initiated by the long photoperiod, the decreasing photoperiod or both. For the delayed implantation, it is speculated that the rapid embryo development followed by the five months of low development of the blastocyst, are induced by either the short photoperiod in December, the increasing day length in January or both (Sempéré et al., 1998). Nevertheless, other deer rut in November and December and they do not have a delayed implantation. Other deer may also feed on more digestible foods in winter, thereby reducing costly activities, such as foraging, and lowering daily cost of gestation and growth. Therefore the reasons for the delayed implantation remain unknown (Sempéré et al., 1998).

Sexual cycle in male roe deer

The gonadotrophin levels in male roe deer are high in three (3) periods per year; January-February, May-June and September-October. The gonadotrophin levels are at their lowest in October-January, when the new antlers are growing. Gonadotrophin stimulates the production of testosterone, and testosterone lowers the gonadotrophin by means of a negative feedback mechanism. Testosterone rises from February until its maximum value in July, during rut, and it decreases in August, being at their lowest in October (Sempéré et al., 1998).
The fact that testosterone depends on increasing and decreasing day length, has been proven by researches who found that roe deer in southern France have an earlier rise in testosterone (starting in February) than roe deer in Germany (starting in April) (Sempéré et al., 1998).

Sexual cycle in female roe deer

Despite of the fact that female roe deer are monoestrous and can only be fertilized for 36 hours, 98% of adult female roe deer are fertilized during this short period (Sempéré et al., 1998). This fertilization takes place in August, after which the egg divides into a blastula within 14 days. The egg is not implanted in the uterus for another 4.5 months, which will not be before early January (Geist, 1998), or, according to other sources, mid December (Goss, 1983). Gestation takes about 300 days, including the 5 months of diapause (Sempéré et al., 1998), which is strange, because due to their small body size, a low gestation period is expected (Geist, 1998). The delayed implantation is believed to be a secondary mechanism of roe deer to adapt to certain environments and weather conditions. This delayed implantation allows the roe deer to mate during favorable season, which is summer, and still be able to give birth in spring, which is the optimal moment for the fawns to survive (Geist, 1998; Huygen & Poutsma, 2000; Sempéré et al., 1998). Normally, progesterone levels are low during the diapause; however, in female roe deer that are not fertilized during the rut a high level of progesterone remains indicating that they do not recycle the corpus luteum and therefore must be monoestrous.
There is no seasonal endogenous rhythm discovered in the metabolic rate of European roe deer, whereas in other animals, seasonal changes in metabolic rate prepare the animal for the winter and for a successful reproduction (Sempéré et al., 1998).

The energy costs of pregnancy in roe deer are comparable to other wild herbivores and increases with 15% during the last two months (Sempéré et al., 1998). Since the fat reserves in roe deer are relatively low, they must provide themselves through a different source of energy during gestation, which could be the food supply. The survival rate of fawns varies greatly between years and seems to have a relation with the amount of able food resources. This suggests that during the periods when food supply is low, females reduce their lactation. Although the high levels of prolactin, lactation does not affect the timing of ovulation in female roe deer (Sempéré et al., 1998).

Many other methods to reduce energy costs are exhibited by other deer. For example the musk deer; this deer makes trails in the snow and uses these trails every time, thereby reducing the costs of locomotion. Roe deer are not known to do this. Musk deer also have less intensive mating activities and they do not grow antlers (Geist, 1998).

Some characteristic features of roe deer (Goss, 1983) (Fig. 5):

- They have 70 chromosomes
- They rut in July and August
- They cast their antlers in October until December
- They grow their antlers in October until February
- They shed their velvets in February until April
- They give birth in May (Goss, 1983; Zoogdiervereniging, 2013)
- The lactation period is 6-10 weeks (Zoogdiervereniging, 2013)

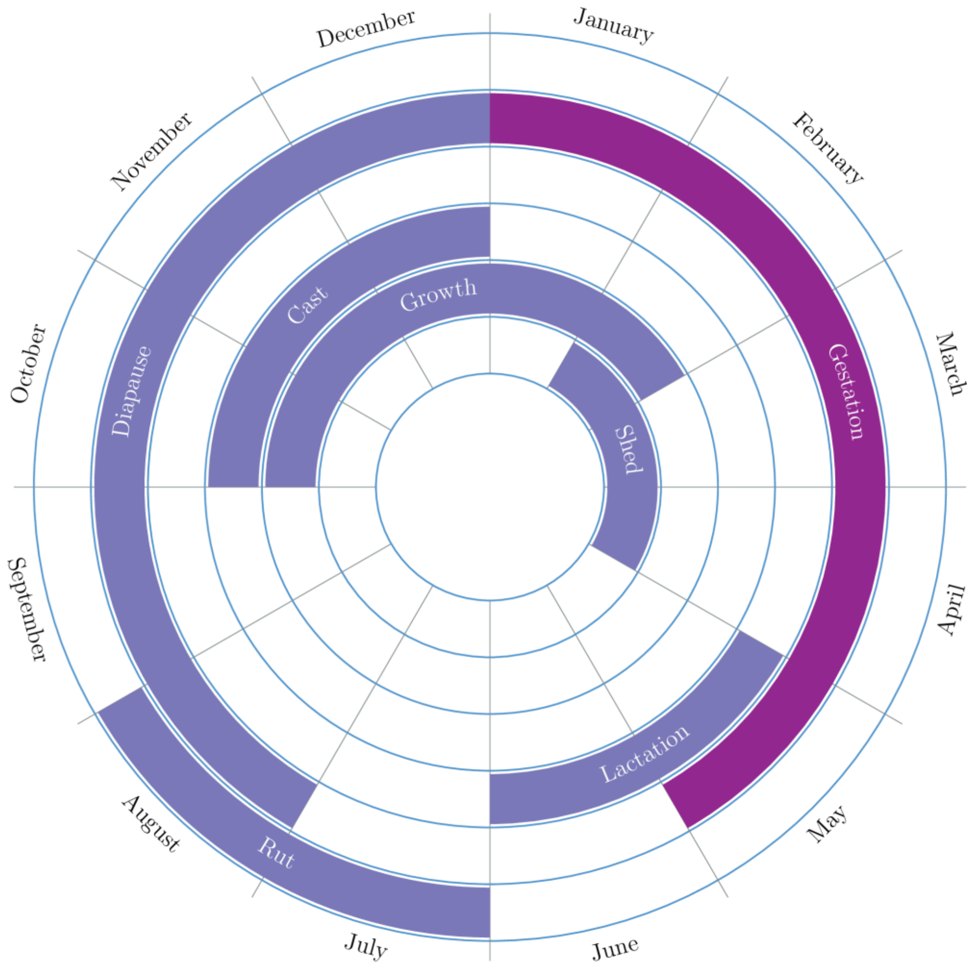

Fig. 5 Life cycle of roe deer

Age determination

Tooth wear is the most used method in the determination of age of roe deer (Brinkman, 2009; Gaillard, Sempéré, Boutin, Laere, & Boisaubert, 1992; Hewison et al., 1999; Lister et al., 1998; Walhström, 1995). Tooth wear, though, is not the most accurate method as it can differ within and among populations and depends on multiple factors such as; diet, habitat and even individual characteristics (Hewison et al., 1999). Besides using tooth wear, few other methods have been described in the use of determination of the age of roe deer. Age determination by other means can be performed, but these are quite expensive in time and costs. For example, the white bands in the cementum of teeth can be used (Aitken, 1975). For this, histological transparent or ground sections have to be made; the transparent sections have to be observed with transmitted light and the ground sections with reflected light (Fancy, 1980).

Therefore, only age determination based upon tooth wear will be discussed in further detail. The dental formula of roe deer can be described as follows (Brinkman, 2009);

$$I\,\frac{0}{3}\,;\ C\,\frac{0}{1}\,;\ \mathbf{P}\,\frac{\mathbf{3}}{\mathbf{3}}\,;\ \mathbf{M}\,\frac{\mathbf{3}}{\mathbf{3}}\ X\ 2 = 32$$

There are no upper canine or incisive and only two canines in the lower jaw. The characteristic two-lobed appearance of the fourth lower molar is created by its cusps which are at the poster lingual and mesio lingual sides of the molar (Lister et al., 1998).

Age determination is done by looking at the premolar and molar of the lower jaw. The young of the year, being younger than one year of age, have three primary premolar and two permanent molars. The juvenile roe deer, being between one and one and a half year of age, will have two permanent premolars, which are P1 and P2. The third premolar, P3, is pushed out by the permanent P3. This permanent P3 is a two-rooted tooth, contrary on the primary P3, which is a three-rooted tooth. From the age of one and a half year onwards, there are 6 permanent teeth (Brinkman, 2009).
Between the age of one and a half year and 5 year, age determination using the amount of tooth wear is rather specific. The premolars are being worn off and the molar will get a ridge-like appearance, being like a saw. After the age of five year, the forepart of the first molar is smooth (Brinkman, 2009).

Age determination by the appearance of the animals can also be done, but this is rather speculative and depends on the observation skills of the ranger. It is based upon the antlers, the physique and the behavior of the roe deer.

Appendix B: Pathogens of Dutch *Capreolus capreolus*

There are many pathogens that can infect roe deer of which almost none is roe deer specific and can infect other cervids and ruminants as well. This is not a complete overview, as roe deer could possibly be infected with many other pathogens not discussed in this section. Only the most common pathogens as named in the analysis will be discussed without their specific details as this is beyond the subject of this study. Most of the other pathogens found in the roe deer submitted for this research will only be mentioned briefly since they have only been found once or twice and are considered of minor importance.
Some of the pathogens discussed below, are only described in foreign countries, but since these countries have close relations with the Netherlands or are bordered to the Netherlands, these parasites are considered to be emerging.

Infectious pathogens among wildlife can play an important role on the health and reproductivity of livestock and they also have a zoonotic potential. Environmental changes can modify the transmission of pathogens, making them even more pathogenic or transmissible. Due to an increasing amount of interactions with wild animals, zoonotic and epizootic infections will rise. Of all identified infections in humans, more than 50% is zoonotic and are multihost pathogens. 77% of the 800 zoönotic diseases are caused by pathogens which also affects wildlife. 90% of the 125 emerging zoönotic diseases affect wildlife, and from the diseases that have emerged in the past 10 years, 75% was of wildlife origin (Billinis, 2013).

Endoparasites

Lungworm infections in wildlife are associated with considerable mortality and morbidity and therefore of major importance. Multiple lungworms of *Dictyocaulus* have been described in the literature, but in the submitted roe deer, only *D. eckerti* of this family has been found. The smaller lungworm, *Varestrongylus capreoli*, is specific for roe deer, as the name suggests (Chowdhury & Alonso Aguirre, 2001).

Dictyocaulus eckerti

Dictyocaulus eckerti has the same appearance as *D. viviparous*, with some minor differences in appearance and is especially important in areas with temperate climates combined with high rainfall (Wall, 2007). In *D. eckerti*, the tertiary larvae are ingested and migrate from the intestine trough lymphatic vessels and the ductus thoracicus into the right heart and the capillaries of the lung. They colonize the bronchioles as fourth-stage larvae and mature there into adult worms. The embryonized ova will be deposited by the females in the bronchioles. After the development in first stage larvae, these larvae will migrate into the pharynx from where they are coughed up of directly swallowed and passes the intestine with the feces. Especially young animals are susceptible for *D. eckerti* and it is known to cause severe inflammations in the respiratory system (Chowdhury & Alonso Aguirre, 2001).

Varestrongylus capreoli

Varestrongylus capreoli, also called *Capreaocaulus capreoli*, has its predilection place in the lungs too (De Bosschere et al., 2007; Wall, 2007). These parasites require an intermediate host, which are slugs and snails. The tertiary larvae develop in the slugs and snails after which they will get ingested while browsing. The larvae can also be ingested directly whilst browsing when they have left the intermediate hosts. They enter the lungs through the so called "lymph-blood" route, after which they will form clusters and start breeding. The first stage larvae will migrate into the pharynx, as in *D. eckerti*, and are coughed up or swallowed.

They are known of causing death at the close of the winter season, due to severe inflammation and total exhausting (Chowdhury & Alonso Aguirre, 2001).

Gastrointestinal worms

Parasitic gastroenteritis is very common among wild ungulates and in free-ranging ruminants it is almost always a mixed infection with different species of *Trichostrongylidae, Strongylidae, Trichonematidal* and *trichurids* (Chowdhury & Alonso Aguirre, 2001). Normally these worms are of little clinical significance, but combined they can give a severe gastroenteritis (Wall, 2007). Various worms of these families have been found in the submitted roe deer, alone or as a combination with others of different families. The following parasites have been found in the submitted roe deer; *O. leptospicularis (*synonym*: O. kolchida* and *Skrjabinagia kolchida* (Wall, 2007)), *Nematodirus spp., N. spathiger, N. europaeus, Haemonchus contortus, Oesophagostomum venulosum, Trichuris ovis, Trichuris globulosa, Trichostrongylus colubriformis, T. capricola, T. axei* and *Capillaria* (Chowdhury & Alonso Aguirre, 2001).

Haemonchus contortus

Haemonchus contortus, also called "barber's pole", has gotten its name due to the appearance of the females. In a fresh specimen, the ovaries appear white and are spirally winded around the intestine, which is filled with blood and appears red. The pathogenesis of *H. contortus* is that of an acute haemorrhagic anaemia, this is due to the blood-sucking habit that these worms have. *H. contortus* has its predilection place on the abomasum and each worm can suck up to 0.05ml of blood per day by ingestion through the lesion they make in the abomasal wall (Wall, 2007).

Oesophagostomum venulosum

Roe deer get infected with *Oesophagostomum venulosum* by ingestion of the third stage larvae whilst grazing. Inside the body, no migration of *O. venulosum* occurs. The fourth stage larvae will attach or enter the wall of the large intestine, which is its predilection place. Generally, *O. venulosum* is considered non-pathogenic and is not associated with clinical signs. *O. venulosum*, like other trichostrongylids, is only present in spring and summer (Sissay et al., 2007; Wall, 2007). There is evidence for the survival of *O. venulosum* in sheep in temperate areas during autumn and winter in which there is a hypobiose with the animal (Wall, 2007).

Trichostrongylids grow up to a third stage in the host feces, after which they migrate into the surrounding vegetation in order to get ingested by a host. In this host the cycle completes itself until the adult stage which causes the inflammation. The eggs will be excreted with the feces after which the cycle restarts (Chowdhury & Alonso Aguirre, 2001).

Other

Moniezia expansa is, together with *M. benedeni*, the only adult tapeworms that can infect free ranging ruminants in Europe. In this research, only one (1) submitted roe deer was found with *M. expansa* and none with *M. benedeni* (Chowdhury & Alonso Aguirre, 2001).

Sertaria cervi is normally only found inside the abdominal cavity and rarely inside organs. Only few reports are known of the clinical manifestations of *S. cervi*. Even though it is a parasite of the abdominal cavity, it can also invade the meninges and the subdural cavity of the spinal cord causing immobility (Chowdhury & Alonso Aguirre, 2001).

In literature, *Fasciola magna* has been described in roe deer in Europe, to cause fatal diseases. It was introduced along with the American deer. *Fasciola hepatica* is not very common described in literature to cause disease in roe deer in the Netherlands or across Europe, however some cases were found in the submitted roe deer (Chowdhury & Alonso Aguirre, 2001). Two (2) other endoparasites have been found in the submitted roe deer. These were: *Ostertagia lasensis* and *Spiculopteragia boehmi*.

Ectoparasites

Ectoparasites rarely cause severe disease, although they can serve as a vector for many other diseases (Radostits, 2007). However, a severe infection with ectoparasites can cause the skin to react, for example with an inflammation or with alopecia (De Bosschere et al., 2007). Besides this, a severe infection causes distress in the animal and the bites can become very serious (Radostits, 2007).

Ixodes ricinus

The main importance of *Ixodes ricinus* is the transmission of various vector-borne pathogens, such as: *Borrelia burgdorferi* and *Anaplasma phagocytophilum* which have been investigated in roe deer, amongst other pathogens and other cervids (Den Boon et al., 2004; Kjelland et al., 2011; Schouls et al., 1999; Wall, 2007; Williams, 2001). *I. ricinus* can also be a vector for *Coxiella brunetti,* the main pathogen of Q-fever (Wall, 2007). *Anaplasma phagocytophilum* and *Coxiella brunetti* will be discussed briefly below in the section "Bacteria". Roe deer are poor hosts for *Borrelia burgdorferi*, since infected ticks have been removed from deer without any symptoms and *I. ricinus* also failed to get infected while feeding on roe deer. However, roe deer are a major host for *I. ricinus* and is part of their survival cycle (Williams, 2001).
Severe infestations with *I. ricinus* can cause anemia and bites can damage the host, leaving a spot with local injury which may predispose to secondary bacterial infections. The site can also predispose for myiasis. However, only one (1) submitted roe deer was found to have a local injury of the skin. None of the above mentioned lesions have been observed, with the exception of anemia (Wall, 2007).

Cephenemyia stimulator

There are four known species of *Cephenemyia* across Europe. These are: *C. trompe, C. stimulator, C. auribaris* and *C. ulrichii.* They are described in different species of deer. *C. trompe* is described in reindeer *(Rangifer tarandus), C. stimulator* in roe deer *(Capreolus capreolus), C. auribaris* in red deer *(Cervus elaphus)* and *C. ulrichii* in moose *(Alces alces)* (Nilssen et al., 2008). Literature describes *C. stimulator* as an obligate parasite that inhabits the throat, pharynx and nasal cavity of cervids, causes no lesions and is generally well tolerated (Nilssen et al., 2008). However, larvae of *Cephenemyia* are described to occasionally cause death by suffocating (Nilssen et al., 2008; Wall, 2007). The general effect of *Cephenemyia* is loss of condition, which is caused by the adult flies which are a major cause of disturbance and the roe deer responses with avoidance, thus leading to reduced food intake and loss of condition. In summer, when the larvae are deposited, keratitis and blindness may occur due to occasionally deposit in the eye (Wall, 2007).
The female fly ejects her larvae in the nostrils of the roe deer whilst flying. The larvae will migrate from the nostrils into the retropharyngeal pouches, were they develop. Developing larvae have a white appearance while the fully developed larvae are yellowish-brown. The larvae are expelled from the throat after development and will pupate in the soil. The adult flies lack a mouth part and are therefore short-living (Department of Natural Resources, 2013; Wall, 2007).

Lipoptena cervi

Females of the *Lipoptena spp.* will land on the roe deer were they deposit the fully developed third stage larvae. The larvae will fall on the ground on which they pupate and become adult flies. Both sexes of the *Lipoptena spp.* are so called blood-feeders (Hodžić, A., Omeragić, J., Alić, A., Jažić, A., Zuko,A., 2012; Wall, 2007).

The main effect of the flies is that they are annoying and cause disturbance in the roe deer (Wall, 2007). They could also induce inflammation, hyperemia and significant blood loss on their host (Hodžić, A., Omeragić, J., Alić, A., Jažić, A., Zuko,A., 2012). *Lipoptena spp.* could also play a part in the transmission of some of the subspecies of the vector-borne pathogen *Bartonella* (Açici et al., Aug2012; Hodžić, A., Omeragić, J., Alić, A., Jažić, A., Zuko,A., 2012).

Other ectoparasites that have been found on the submitted roe deer, but of minimal significance, were; *Oestrus ovis, Anoplura, Smithurinus spp.* (which is not a parasite, but an insect that happened to be present), *Damalinia meyeri, Hippobosca equine* and *Hypoderma diana* (which is actually a parasite of the connective tissue (Radostits, 2007; Wall, 2007)).

Protozoa

Toxoplasma gondii is one of the zoönotic pathogens described in wild roe deer in Europe. Seroprevalences of *T. gondii* in wildlife are known for a variety of European countries such as; Belgium, Hungary, Sweden, Norway, United Kingdom and Ireland. Roe deer with sufficient antibodies titers are found in Spain and France (Aubert et al., 2010). Toxoplasmosis has been reported in red deer, roe deer, in moose and in fallow deer in Spain, France, Norway, Italy, the Czech Republic, Sweden and Portugal (Billinis, 2013).

Neospora caninum is closely related to *T. gondii*; it is a protozoan parasite with a worldwide distribution. Neosporosis has been reported in red deer, in roe deer, in Spain, the Czech Republic and Sweden. *T. gondii* and *N. caninum* antibodies have been detected in blood samples from various *Cervidae* species in Greece (Billinis, 2013).

Another parasite in this family is *Sarcocystis*. Various different subspecies have been reported in roe deer, but also in other types of deer. *Sarcocystis* derives its name from its intramuscular stage, the cyst, which is present in the intermediate host. The intermediate hosts are species-specific for different species of *Sarcocystis* (about 130 different species have been reported). The *Sarcocystis* as found in the final hosts are family-specific (Wall, 2007).

Bacteria

No roe deer specific bacteria have been found to be reported in literature. Roe deer can get infected with all kinds of bacteria with which other animals could also get infected. Some of the bacteria found in Dutch roe deer during this research are; *Escherichia coli, Campylobacter spp., Clostridium perfringens, Mannheimia haemolytica, Fusobacterium necrophorum* and *Actinobacillus lignieresii.* Other bacteria found in literature to affect roe deer are; *Salmonella spp.* (Williams, 2001), *Mycobacterium tuberculosis* (frequently found in roe deer in Spain, France, Slovakia and the Czech Republic (Billinis, 2013)), *Anaplasma phagocytophilum* (has been reported in Spain, Poland, Italy, Slovenia, Austria, Denmark and Norway (Billinis, 2013; de la Fuente et al., 2008)) and *Coxiella brunetti* (Billinis, 2013; Rijks et al., 2011), among others.

Viruses

Ovine herpesvirus 2 is the only virus found in the submitted roe deer. These were two (2) occasional cases. It has also been found in German roe deer (Frolich, Li,

& Muller-Doblies, 1998). Other viruses described in roe deer are Bluetongue, this was not described in Dutch roe deer, but it has been reported in roe deer in Spain and Belgium (Billinis, 2013). Also described is *Deer herpesvirus*, this is a herpesvirus which is closely related to *ovine herpesvirus 2* (Radostits, 2007). Two more viruses have been described; *parainfluenza virus 3*, for which serologic and virologic evidence has been reported and pestiviruses in northern Germany (Williams, 2001). Antibodies, viruses and lesions have been found in free living roe deer all across Europe.

Other

Actinomyces is a fungus which has been found in roe deer in this research, but it is not a roe deer specific pathogen. Hyperplasia of the white pulp of the spleen is not a pathogen, but will be discussed in this section due to its relevance for this research. The normal foci of the white pulp of the spleen are usually not visible on cut section. However, as a result of an antigenic stimulus or a neoplastic process, these foci can become enlarged and can become visible on cut section (McGavin, 2007).

In literature, a distinction has been made between "storage spleen" and "defense spleen". With storage spleen is meant; a spleen that has a high percentage of smooth muscles in the trabeculae and capsules, thus allowing the spleen to expand and contract. This expansion allows the spleen to store a high amount of erythrocytes. The "defense spleens" do not have this capability and mainly serve as a component of the immune system that produces antibodies. The white pulp in storage spleens may have an enlarged appearance due to the contraction of the red pulp. This contraction happens either under the influence of the autonomic nervous system, during hypovolemic shock or during cardiogenic shock. All domestic ruminants and pigs have intermediate spleens, which is a combination of the above described types of spleens (McGavin, 2007).

The presence of iron is the last to discuss in this section, although it is not a pathogen nor directly linked to a pathogen. It is one of the most common found etiologies in the submitted roe deer and therefore considered a relevant etiology in this research. As mentioned, this presence of iron is not defined to one organ or cell type. It can be found inside macrophages or inside 'normal' cells of the organ. It can also be found in many different organs. This iron can be either ingested or it could be the result of a hemolytic crisis, such as the breakdown of erythrocytes (McGavin, 2007). There is a difference between iron inside cells and iron not inside cells as iron could be stored in two forms; hemosiderin and ferritin. Hemosiderin is formed from intracellular aggregates of ferritin, which is found in liver, spleen, bone marrow and skeletal muscle (McGavin, 2007). However, no literature was found to prove any clinical relevance of the presence of iron in roe deer or in small ruminants (Ammerman et al., 1967; McGavin, 2007; van Ravenswaay, Henry, & Ammerman, 2001; Wall, 2007).

Appendix C: Overview of pathological findings contributing to the death of the animal

Topography	Morphology	Etiology
Cardiovascular and hematopoetic systems	**Anemia (N=7)**	Blood sucking parasites (N=6)
	Atrophy of bone marrow (N=1)	
	Erythroid hyperplasia (N=1)	
	Unsufficient erythropoesis (N=1)	
	Myocarditis (N=6)	Parasites not specified (N=1)
	Dilatation of right ventricle (N=3)	
	Degeneration of heart muscle (N=1)	
	Heart failure (N=2)	
	Fibrosis of pericard (N=1)	
	Siderofibrosis (N=1)	
	Lymphadenitis (N=6)	*Clostridium spp.* (N=1), Bacteria not specified (N=1)
	Plasmocytoma (N=1)	
Systemic, Skin, Muscle and Bones	**Lesions consistent with multiple parasitic infections (N=21)**	*Ixodes ricinus* (N=20), *Lipoptena cervi* (N=8), *Damalinia meyeri* (N=4), *Cephenemyia stimulator* (N=2), *Hypoderma diana* (N=1), *Hippobosca equina* (N=1)
	Cachexia (N=19)	Gestation (N=5), *Ixodes ricinus* (N=4), *Dictyocaulus eckerti* (N=4), Parasites not specified (N=4), *Cephenemyia stimulator* (N=1), *Fasciola hepatica* (N=1), Hemolysis (N=1), Polyarthritis (N=1)
	Traumatic lesions consistent with a shot wound (N=116)	Trauma (N=1), Cachexia (N=1)
	Fractures (N=14)	Collision (N=8)
	Systemic hyperemia (N=1)	Shock (N=1)
	Blood loss (N=3)	Collision (N=1), Process in liver (N=1)
	Tumors (N=2, Osteoma=1, osteosarcoma=1)	
	Myositis (N=1)	
	Muscular fibrosis (N=1)	Bacteria (N=1)
	Inflammation (Skin=1, Skeleton=2, Limbs=1)	Bacteria (N=1)
	Dermatitis (N=2)	*Ixodes ricinus, Lipoptena cervi, Linognathus spp.* (N=2)
	Panniculitis (N=1)	
	Alopecia (N=2)	*Ixodes ricinus* and *Lipoptena cervi* (N=2)
	N/A	Collision (N=33), Found dead (N=15), *Ixodes ricinus* (N=5), Parasites not specified (N=3), *Lipoptena cervi* (N=2), Bony mass compressing cerebral tissue (N=1), *Damalinia meyeri* (N=1), *Hypoderma diana* (N=1), , *Linognathus spp.* (N=1)
Nervous system and	**Meningitis (N=12)**	*Mannheimia haemolytica* (N=1)
	Encephalitis (N=6)	Bacteria (N=1)

eyes	Meningo-encephalitis (N=2)	*Ovine Herpesvirus 2* (N=2)
	Hemorrhages in meninges (N=3)	
	Hemorrhages in brain (N=2)	
	Neuritis (N=2)	*Mannheimia haemolytica* (N=1)
	Aplasia of cerebellum (N=2)	
	Blindness (N=1)	
	Ependymoma (N=1)	
	Hydrocephalus (N=1)	Congenital (N=1)
	Abnormal Purkinjecells (N=1)	
	Fibrosis of cornea (N=1)	Bacteria (N=1)
	Inflammation of corpus cilliare (N=1)	
	Ophthalamitis (N=2)	
	Panophthalmia (N=2)	Trauma (N=1)
	Uveitis (N=2)	Bacteria (N=1)
Alimentary system and Liver	**Lesions consistent with multiple parasitic infections (N=44)**	*Haemonchus contortus* (N=31), *Trichuris ovis* (N=10), *Oesophagostomum venulosum* (N=8), *Ostertagia leptospicularis* (N=7), *Spiculopteragia boehmi* (N=5), *Setaria cervi* (N=3), *Trichostrongylidia* (N=3), *Eimeria spp.* (N=1), *Ostertagia lasensis* (N=1), *Skrjabinagia kolchida* (N=1), *Trichuris globulosa* (N=1), *Capillaria* (N=1), *Nematodirus europaeus* (N=1)
	Cachexia (N=22)	*Haemonchus contortus* (N=22), *Trichuris ovis* (N=8), *Oesophagostomum venulosum* (N=6), *Trichostrongylidia* (N=2), *Ostertagia leptospicularis* (N=1), *Trichostrongylus axei* (N=1), Parasites not specified (N=1), Diarrhea (N=1)
	Abomasitis (N=21)	*Haemonchus contortus* (N=7), Trichostrongylidia (N=3), Parasites not specified (N=2), *Ostertagia lasensis* (N=1), *Spiculopteragia boehmi* (N=1), *Oesophagostomum venulosum* (N=1), *Skrjabiniagia kolchida* (N=1), *Campylobacter* (N=1)
	Enteritis (N=8)	*Trichostrongylidia* (N=1), *Trichuris ovis* (N=1), *Oesophagostomum venulosum* (N=1), Parasites (N=1), *Nematodirus spathiger* (N=1), *Clostridium perfringens* (N=1)
	Iliitis (N=1)	
	Colitis (N=1)	
	Gastro-enteritis (N=1)	
	Adhesion of rumen (N=1)	
	Rumenitis (N=1)	Intra-epithelial yeast (N=1)
	Peritonitis (N=3)	
	Pyoabdomen (N=1)	Coccidia (N=1)
	Diarrhea (N=12)	*Haemonchus contortus* (N=3), *Trichuris ovis* (N=2), *Clostridium perfringens* (N=2), *E. coli* (N=1), Bacteria not specified (N=1), *Strongylus* (N=1), Enteritis (N=1), Colitis (N=1)
	Hernia diafragmatica (N=1)	Collision (N=1)

	Hepatitis (N=5)	*Fasciola hepatica* (N=1), *E. coli* (N=1)
	Cholangiohepatitis (N=3)	*Fasciola hepatica* (N=1), *E. coli* (N=1)
	Cholangitis (N=3)	Presence of iron (N=1)
	Fibrosis (N=2)	*Fasciola hepatica* (N=1)
	Hepatic congestion (N=1)	
	Necrosis of the liver (N=1)	
	Liver abscesses (N=1)	Bacteria (N=1)
	Glossitis (N=6)	*Actinomyces* (N=2), Bacteria not specified (N=1)
	Esophagitis (N=1)	Bacteria (N=1)
	Missing elements (tooth) (N=1)	
	N/A	*Haemonchus contortus* (N=3), *Oesophagostomum venulosum* (N=2), *Spiculopteragia boehmi* (N=2), Presence of iron (N=2), *Sertaria cervi* (N=1), *Clostridium spp.* (N=1), *Ostertagia leptospicularis* (N=1), Intestinal worms not specified (N=1)
Respiratory system	**Pneumonia (N=44)**	*Dictyocaulus eckerti* (N=30), Bacteria not specified (N=2), *Varestrongylus capreoli* (N=5), *Campylobacter* (N=1), *Arcanobacterium pyogenes* (N=1)
	Rhinitis, laryngitis, sinusitis, tracheitis (N=1)	*Cephenemyia stimulator* (N=1)
	Laryngitis (N=2)	
	Trauma larynx (N=1)	Dog bite (N=1)
	Bronchopneumonia (N=28)	*Dictyocaulus eckerti* (N=17), *Varestrongylus capreoli* (N=2), Lungworms not specified (N=3), Bacteria not specified (N=3)
	Interstitial pneumonia (N=12)	*Dictyocaulus eckerti* (N=5), *Varestrongylus capreoli* (N=2), Lungworms not specified (N=2)
	Interstitial bronchopneumonia (N=2)	
	Bronchitis (N=2)	
	Broncho-pleuropneumonia (N=1)	Aspiration pneumonia, *Dictyocaulus eckerti* (N=1)
	Pleuro-pneumonia (N=1)	
	Pleuritis (N=6)	*Dictyocaulus eckerti* (N=4)
	Aspiration pneumonia (N=2)	
	Edema (N=1)	
	Fibrosis (N=1)	
	Tumors (N=2, adenocarcinoma=1, anaplastic carcinoma=1)	
	Choking (N=1)	
	Lesions consistent with multiple parasitic infection (N=19)	*Dictyocaulus eckerti* (N=7), *Cephenemyia stimulator* (N=13)
	N/A	*Dictyocaulus eckerti* (N=3), *Cephenemyia stimulator* (N=2)
Endocrine system	Carcinoma of thyroid gland (N=1)	
	Hyperplasia of thyroid gland (N=1)	Calcium and iron accumulations (N=1)

Reproductive system	Omphalophlebitis (N=1) Anomalia preputium (N=1) Dystocia (N=1) Placentitis (N=1)	
Urinary tract	Interstitial nephritis (N=2) Nephritis (N=2) Renal necrosis (N=2)	 Bacteria (N=1) Ischemia (N=1)

Appendix D: Overview of “other” pathological findings

Topography	Morphology	Etiology
Cardiovascular and hematopoetic systems	**Hyperplasia of white pulp of the spleen (N=147)**	
	Lymphadenitis (N=5)	
	Presence of iron (N=34)	
Systemic, Skin, Muscle and Bones	**Fractures (N=4, of which 3 healed)**	
	Thickening of epidermis (N=2)	
	Lesions consistent with multiple parasitic infection (N=1)	*Ixodes ricinus* (N=1),
	Pustule (N=1)	
	Inflammation (Skeleton=1)	Trombi (N=1)
	N/A	*Sarcocystis* (N=136), *Ixodes ricinus* (N=92), *Lipoptena cervi* (N=47), *Linognathus spp.* (N=15), *Damalinia meyeri* (N=12), Prescence of iron (N=11), *Hypoderma diana* (N=5), *Smithurinus spp.* (N=1), Parasites on skin not specified (N=1), *Cephenemyia stimulator* (N=1), *Dictyocaulus eckerti* (N=1)
Nervous system and eyes	**Meningitis (N=3)**	
	Encephalitis (N=3)	Bacteria (N=1)
	Hemorrhages in meninges (N=3)	
	Melanine pigment (N=1)	
	Hyperemia (N=3, of cerebrum N=2, of plexus choroideus N=1)	Bacteria (N=1)
	Conjuctivitis (N=1)	Bacteria (N=1)
	Cataract left eye (N=1)	
	Keratitis (N=1)	
	Uveitis (N=1)	Old trauma (N=1)
	Psammomateus meningioma (N=1)	
Alimentary system and Liver	**Enteritis (N=7)**	
	Abomasitis (N=5)	*Haemonchus contortus* (N=1), Parasites not specified (N=1)
	Glossitis (N=4)	*Sarcocystis* (N=1)
	Cachexia (N=1)	
	Colitis (N=2)	
	Fibrosis (N=2)	
	Peritonitis (N=3)	*Trichostrongylidia* (N=1)
	Hepatitis (N=2)	*Coccidia* (N=1)
	Cholangitis (N=1)	Presence of iron (N=1)
	Hernia diafragmatica (N=1)	Extern blunt trauma (N=1)
	Coccidiosis (N=1)	
	Prescence of iron (N=58)	
	N/A	*Haemonchus contortus* (N=7), *Trichuris ovis* (N=7), *Strongylus* (N=5), *Oesophagostomum*

		venulosum (N=3), Bacteria not specified (N=4), *Clostridium spp.* (N=2), *Coccidia* (N=2), *E. coli* (N=2), *Sertaria cervi* (N=2), *Ostertagia leptospicularis* (N=2), *Spiculopteragia boehmi* (N=2), *Trichostrongylus capricola* (N=2), *Moniezia expansa* (N=1), *Nematodirus europaeus* (N=1), *Ostertagia lasensis* (N=1), *Ostertagia kolchida* (N=1), *Capillaria* (N=1), *Trichostrongylidia* (N=1), *Campylobacter* (N=1), *Actinomycose* (N=1)
Respiratory system	**Pneumonia (N=21)**	*Dictyocaulus eckerti* (N=9), Lungworms not specified (N=4), *Varestrongylus capreoli* (N=1), Bacteria not specified (N=1)
	Bronchopneumonia (N=7)	*Dictyocaulus eckerti* (N=3)
	Interstitial pneumonia (N=6)	*Dictyocaulus eckerti* (N=2), *Varestrongylus capreoli* (N=1)
	Bronchitis (N=1)	*Dictyocaulus eckerti* (N=1)
	Edema (N=1)	
	Fibrosis (N=2)	
	Pleuritis (N=2)	
	Hyperemia (N=1)	
	Lesions consistent with multiple parasitic infection (N=2)	*Cephenemyia stimulator* (N=2)
	N/A	*Cephenemyia stimulator* (N=20), *Dictyocaulus eckerti* (N=6), *Oestrus ovis* (N=2), *Varestrongylus capreoli* (N=2), Lungworms not specified (N=1), *Hypoderma diana* (N=1)
Reproductive system	Cellulitis on epididymis (N=1)	
	Presence of iron (N=1)	
Urinary tract	Nephritis (N=4)	
	Renal necrosis (N=2)	
	Fibrosis (N=1)	
	Presence of iron (N=19)	

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