Infectious diseases in the European brown hare (*Lepus europaeus*) in the Netherlands

An analysis of past and present study, a contribution to future studies

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Abstract

The European brown hare (*Lepus europaeus*, Pallas 1778) is an important game animal in the Netherlands and Europe. A decline in its population has been seen in Europe for several decades, and has also been noticed in the Netherlands. Diseases have been linked to this decline, but it is unknown to which extent. Therefore, the aim of this study was to increase the number of submissions, to make an analysis of infectious diseases, to investigate the role of reproductive life cycle in susceptibility to diseases, and to create a hare specific examination protocol. In order to do so, a pilot was used. Hare reports from 2008-2011 were analyzed, during present and retrospective study, and a literature study was performed. In total, 56 hares were submitted, of which 54 hares could be used for analysis. The most morphological diagnoses were identified in the respiratory system, with pneumonia most commonly found. Etiological diagnoses could be made of diseases in different systems, which could be found in 59% of the hares. Pasteurellosis was identified in 11%, *Staphylococcus* spp. in 9% and yersiniosis in 2%. Only 1 out of 54 hares was suspected of having a virus, the European Brown Hare Syndrome virus. Ticks and *Eimeria* spp. were the ecto- and endoparasites most found. Amyloidosis was found in 8 out of 54 hares. Two separate protocols were created, designed to serve as an instruction and reminder. The scale of this study was limited, even though the pilot led to more cases. The analysis of infectious diseases was incomplete due to the lack of etiological diagnoses. A relation was found between the reproductive life cycle and diseases, but previous studies also showed that weather conditions can contribute to this relationship. The necropsy and sampling protocol and the other results from this study can contribute to future studies on infectious diseases in the European brown hare in the Netherlands.

Acknowledgement

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Introduction

The European brown hare (*Lepus europaeus*, Pallas 1778) is a species in the family of Leporidae and of the Order of Lagomorpha. It has 16 subspecies (Hoffman, Smith 2005). Over time it has grown into an important game animal in the Netherlands and Europe (Flux, Angermann 1990). It has also been introduced in other parts of the world, and therefore it has a worldwide distribution, as can be seen in figure 1.

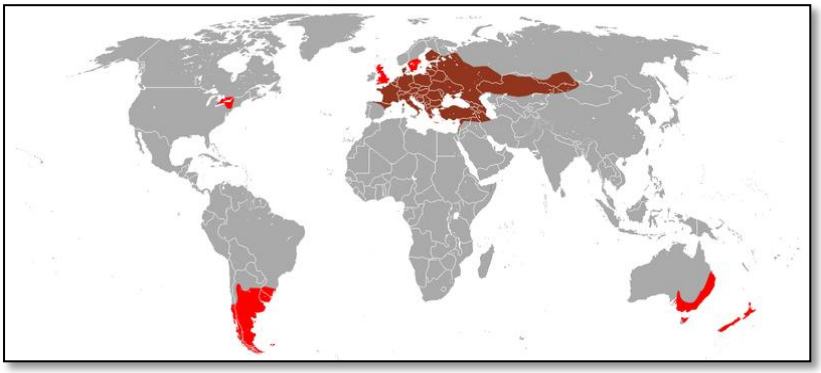


Figure 1 *A map of the distribution of the European brown hare. Native: dark red, introduced: red(Smith, Johnston 2008a).*

Other hare residents in Europe include the broom hare (*Lepus castroviejoi*) in Spain; the mountain hare (*Lepus timidus*), which is found in Scandinavia, isolated populations in Ireland and Scotland, and has been introduced in England (Flux, Angermann 1990); the Corsican hare (*Lepus corsicanus*), which has a small range of distribution in Sicily (Italy) and is suspected on the French island of Corse (Angelici et al. 2008); and the Iberian hare (*Lepus granatensis*) which exists in Portugal and Spain and was introduced in France (Smith, Johnston 2008b). The European brown hare, hereinafter also referred to as 'hare', is the only hare found in the Netherlands.

Hare populations have been declining in Europe since the 1960s (Smith et al. 2005). In the Netherlands there has been a slight decline in hares killed during hunting season. In the period 2006-2008 the average was 6.8 hares per 100 hectare. In comparison, in 1980 this was approximately 8 hares per 100 hectare. The amount of hares shot during hunting season is accounted for approximately 30-40 % of the total hare population during autumn in the Netherlands (Montizaan, Siebenga 2010). The hare decline in Europe has been linked to intensified agriculture (Flux, Angermann 1990; Frölich et al. 2003; Wibbelt, Fröhlich 2005; Reichlin et al. 2006). The rise of mechanized agriculture and the use of chemicals are most likely contributors. However, in Denmark, Germany and Poland, high densities have been reached under intensive agriculture (Flux, Angermann 1990). Other external factors that have been linked to the declining hare populations in Europe include predators, animal and human, climate change and, last but not least, diseases (Reichlin et al. 2006; Marboutin, Peroux 1995; Harcourt-Brown, Whitwell 2003).

Certain infectious diseases, like European Brown Hare Syndrome (hereinafter abbreviated to EBHS), pasteurellosis, yersiniosis, and coccidiosis, can cause high mortality rates and may therefore affect hare populations (Wibbelt, Fröhlich 2005). But also toxoplasmosis and, more recently, tularemia have been reported causing mortality in local hare populations (Sedlak et al. 2000; Decors et al. 2011). The occurrence of an external factor, like disease, can possibly be influenced by the reproductive life cycle, an internal factor. Different stages in this cycle may mean different susceptibility to infectious diseases. It is still uncertain to which extent disease plays a role in the decline of hare populations.

This study was performed in order to gain insight into infectious diseases in hares in the Netherlands and to contribute to future studies. Therefore, the aim of this study was to (i) increase the number of submissions by means of a pilot; (ii) make an analysis of infectious diseases in hares in the Netherlands; (iii) examine the influence of the reproductive life cycle on the susceptibility to diseases; and (iv) set up a hare specific examination protocol.

Materials and Methods

A pilot was started early September 2011 by the Dutch Wildlife Health Center (DWHC), located in Utrecht. By means of a brochure, hunters and game managers from six different areas in the Netherlands were asked to submit hares found dead and hares shot during hunting season, which occurred diseased. Hunting season started October 15th and ended December 31st, 2011. The six different Dutch areas included four areas from the provinces of Drenthe, Overijssel, and Limburg, which were chosen based on previous reported problems in 2011. The other two areas served as control groups and were situated in the province of Utrecht.
The present study (internship) started on November 7th and ended on December 31st, 2011. During this time, 14 hares were submitted and were kept cool until post mortem examination was performed. During necropsy, a standard protocol for examination and sampling was followed, as stated in Annex 1. Tissue samples for histopathology were taken, which were preserved in 4% buffered formaldehyde and then fixated in paraffin. After necropsy, an additional standard DWHC sampling protocol for preservation (-80°C) was followed. These tissue samples included blood, bone marrow, brain, gonad, heart, intestine, kidney, liver, lung, spleen, stomach, and uterus. Further examination such as bacteriology, virology or parasitology was performed when this was found necessary by the pathologist during necropsy or histopathology.
In addition, tissue samples from the spleen and lung were taken and sent to the Central Veterinary Institute (hereinafter abbreviated to CVI) in Wageningen, to identify the bacterium *Francisella tularensis*, which causes tularemia.
To extend the quantity of hares for analysis, a retrospective study was also performed. This included 42 DWHC hare reports, present in the archives of the Veterinary Pathological Diagnostics Centre (hereinafter abbreviated to VPDC), with necropsies dating from February 11th, 2008 till November 1st, 2011.
A schematic overview of all 56 cases, present and retrospective study, was made (see Annex 2). General information such as 'necropsy number', 'necropsy date', 'province', 'sex', 'age', 'weight' and 'way of submission' were directly obtained from report information. Body condition, noted in the reports, was based on proportions of fat, among others subcutaneous and mesenterial, and muscles. For the overview, this was analyzed and categorized as 'poor', 'moderate', 'severe' or 'cachectic'. To specify the morphological diagnosis, each report was inspected and analyzed by a pathologist in order to exactly establish the morphological diagnosis in each hare. These morphological diagnoses were arranged in groups based on 'organ', 'degree', 'progress' and 'distribution'. When hares were submitted with trauma being the cause of death, it was also mentioned in 'morphological diagnosis'. The presence of ectoparasites, endoparasites, other notable information, and negative test results were noted based on report information. When no morphological diagnosis could be found, the morphological diagnosis: 'presence of coccidiosis' or 'presence of amyloidosis', if present, were noted. As mentioned before, these results were normally only noted in 'endoparasites' or 'other'.
If an etiological diagnosis was made or suspected, it was noted. If not, 'nothing found' or 'no further examination done' was noted. Also, if no etiological diagnosis was made and trauma was the cause of death, 'trauma' was noted. Although the last three are no etiological diagnoses, they were included for the completeness of the overview.
Literature study was performed to obtain information about the reproductive life cycle of hares and to obtain information about its role in susceptibility to diseases. Based on literature study, an overview of the most important differential diagnoses in the European brown hare was made (see Annex 3). This overview and the participation in necropsies and sampling, were used to create a hare specific examination protocol.

Results

Pilot

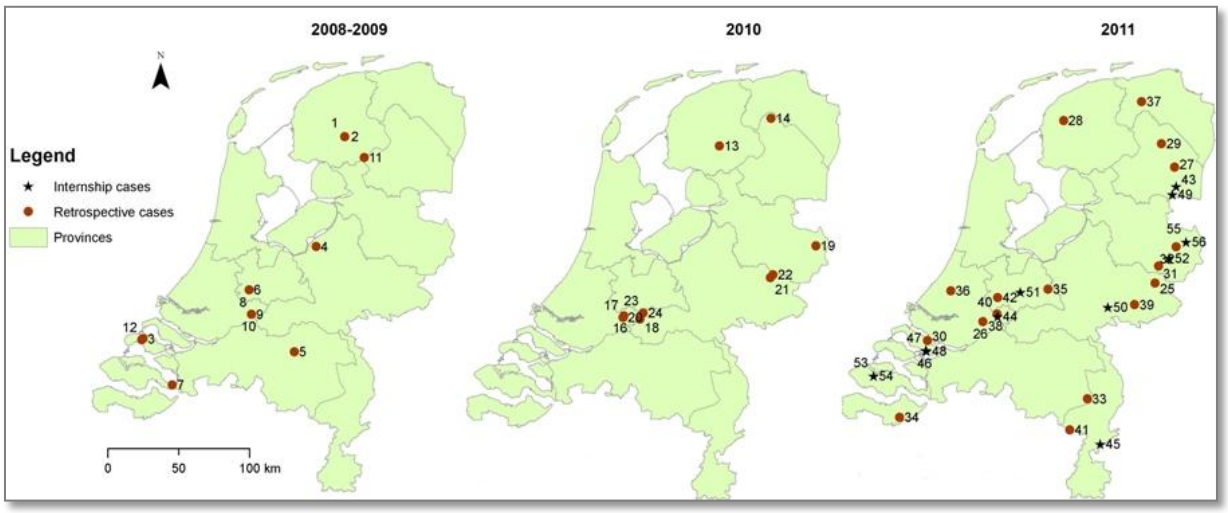


Figure 2 *An area overview of the 56 hares submitted during 2008-2009: 12; 2010: 12; and 2011: 18 & 14 (present study).*

There was an overall increase of cases in 2011, as can be seen in figure 2. During the pilot, 22 hares were submitted, of which 14 cases were submitted during the present study.
As shown in figure 3, the three provinces with problem areas (Limburg, Drenthe, Overijssel) showed a notable increase of two, three and four cases in 2011 compared to 2010. Although it served as a control group, Utrecht also showed an increase, with only one case in 2009, zero cases in 2010 and three cases in 2011. No brochure was circulated in these provinces, but Gelderland, Zuid-Holland and Groningen also showed an increase compared to previous years. Every year, Zuid-Holland has been the highest contributor. During 2008-2011, no hares from Noord-Holland and Flevoland were submitted.

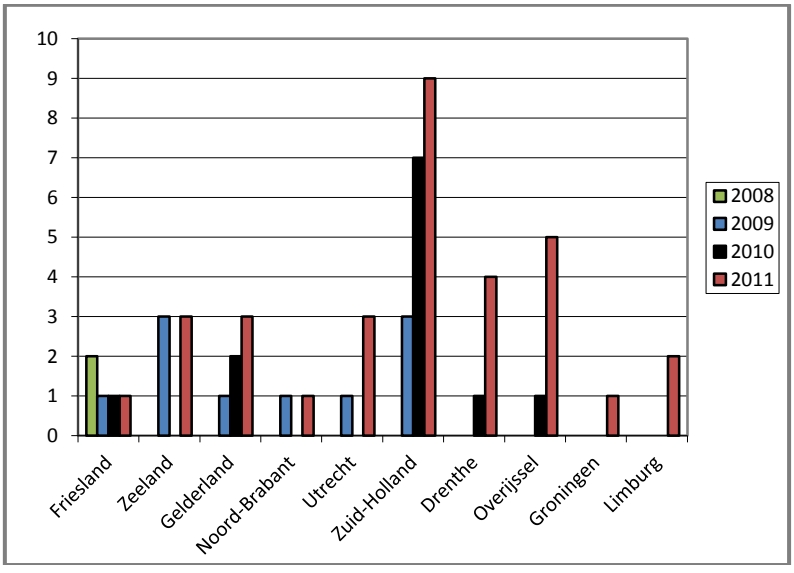
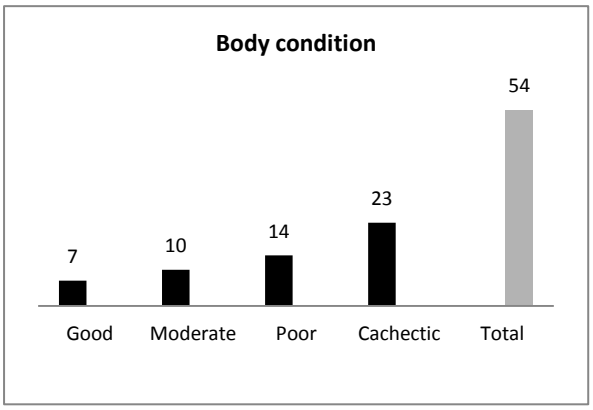


Figure 3 *The hares submitted, from 10 provinces, during 2008, 2009, 2010 and 2011.*

Hare reports

Out of the 56 hares submitted, only 54 hares could be used for data collection. Two hares could not be used due to severe autolysis and abnormal state (used as bait). Juvenile and adults were not noted consistently, thus no analysis relating to age could be performed in this matter.



Body condition is shown in figure 4. Good body condition was found in 7 out of 54 hares, although all hares were submitted as being diseased. Still the majority of the hares was in a poor body condition or even stated as cachectic.

Figure 4 *Body condition in 54 hares.*

The hares used for data collection often had more than one morphological diagnosis which could occur in different systems. A distinction has been made between a morphological diagnosis with an etiology and a morphological diagnosis without an etiology, but with an etiological diagnosis made in another system.
An overview of the systems with the most morphological diagnoses, their etiology or other etiological diagnosis is shown in table 1.

System	Morphological diagnosis	Etiology	Other etiological diagnosis
Respiratory (n=21)	Pneumonia (n=6/12)	*Pasteurella* sp & *Pseudomonas luteola* (1), *Pasteurella* sp. (11), *Staphylococcus aureus* (32)	**Cutaneous**: Streptococci and Staphylococci (44), *Staphylococcus aureus* (49), *Staphylococcus pseudointermedius* (51)
	Bronchopneumonia (n=8/8)	*Pasteurella* sp. (5), Gram negative bacilli (non-fermenter) (12), *Mannheimia hemolytica* (13), *Pasteurella multocida* (31,43), Lungworms (18,23,24)	-
	Tracheitis (n=0/1)	-	-
Cutaneous/ conjunctival (n=12)	Dermatitis (n=6/8)	*Bordetella* sp. (7), Streptococci and Staphylococci (44), *Staphylococcus aureus* (49,53)	**Respiratory**:*Mannheimia hemolytica* (13), *Pasteurella multocida* (43)
	Conjunctivitis (n=1/1)	Enterobacter spp. & Citrobacter spp. (10)	-
	Conjunctivitis /blepharitis (n=1/1)	Cocoid Gram positive bacteria (9)	-
	Ophtalmitis (n=2/2)	Coccoid bacteria (15), *Staphylococcus aureus* (53)	-
Hepatic (n=12)	Hepatitis (n=1/4)	*Yersinia pseudotuberculosis* (27)	-
	Periportal fibrosis (n=1/4)	-	**Cutaneous**: Streptococci & Staphylococci (44)
	Hepatocellular necrosis (n=1/3)	Suspected EBHS (36)	-
	Liver fibrosis (n=0/1)	-	-
Seromembranous (n=9)	Pericarditis (n=3/3)	-	**Respiratory**: *Pasteurella* sp. & *Pseudomonas luteola* (1), Lungworms (24), *Staphylococcus aureus* (32)
	Pleuritis (n=2/3)	-	**Respiratory**: *Pasteurella* sp. & *Pseudomonas luteola* (1), *Staphylococcus aureus* (32)
	Peritonitis (n=1/2)	-	**Genital**: Extra-uterine pregnancy (17)
	Pericard fibrosis (n=1/1)	-	**Cutaneous**: Streptococci and Staphylococci (44)
Alimentary (n=9)	Jejuno-enteritis (n=1/1)	*Eimeria* spp. (4)	-
	Enteritis (n=6/7)	*Eimeria* spp.(21, 37, 39, 40, 42)	**Other**: Extra-uterine pregnancy (17)
	Gastritis (n=1/1)	*Graphidium strigosum* (19)	-
Lymphoid (n=5)	Lymfadenitis (n=3/4)	*Yersinia pseudotuberculosis* (27)	**Alimentary**: *Eimeria* spp.(4, 39)
	Splenitis (n=1/1)	*Yersinia pseudotuberculosis* (27)	-

Table 1 *An overview of the systems with the most morphological diagnoses, including their/or other etiological diagnoses. Behind the etiological agents, the hare numbers are given.*

The respiratory system showed the highest amount of morphological diagnoses. The most common morphological diagnosis was pneumonia, which was found in eleven hares (one hare had two kinds of pneumonia). Bronchopneumonia had the highest percentage of etiological diagnosis, 100% (8 out of 8), all in the respiratory system.
Seromembranous morphological diagnoses were mostly found with a respiratory etiological diagnosis. Every pericarditis was related to a respiratory etiological diagnosis. This was also the case for 2 out of 3 pleuritis, and although in the third no etiological diagnosis was made, this pleuritis was found in a hare with, among others, pneumonia. Pericard fibrosis had a cutaneous etiological diagnosis, but this hare did also have a pneumonia. The only etiology found in the lymphoid system was *Yersinia pseudotuberculosis* and it was associated with lymphadenitis and splenitis, but also with hepatitis. There was also a presence of bacteria in the adrenal gland, most likely being the same agent. Necropurulent lymphadenitis was found twice in combination with enteritis, and etiology *Eimeria* spp. One hare had a cervical necropurulent lymphadenitis with no etiology found.
One hare had peritonitis which was secondary to an endometritis. The other case with peritonitis also had hepatocellular necrosis, periportal liver fibrosis and pneumonia.
Although a number of anamneses mentioned pus fluids in the eyes, only five hares were diagnosed with an eye disease, one of which was due to a corpus alienum. That is why it was not included in table 1. The etiology of the conjunctival morphological diagnoses comprising only bacterial agents.
The hepatitis cases mostly showed a necropurulent character. Notable were the eosinophil inclusion bodies which were found in one hare with hepatocellular necrosis, it was therefore suspected of EBHS. All cases with periportal and liver fibrosis also showed bile duct proliferation.
Most of the morphological diagnoses in the alimentary system were due to a parasitic infection,
7 out of 9. Furthermore, nephritis was found three times. One was suspected to be caused by *Encephalitozoon* spp., while the other two cases had no renal etiological diagnosis.
There were two cases with endometritis, one could be explained by an extra-uterine pregnancy and this also caused a peritonitis. In the other case of endometritis, an etiology was not found, as well as in one case of encephalitis.
The etiological diagnoses were categorized in ten categories, as can be seen in figure 5.

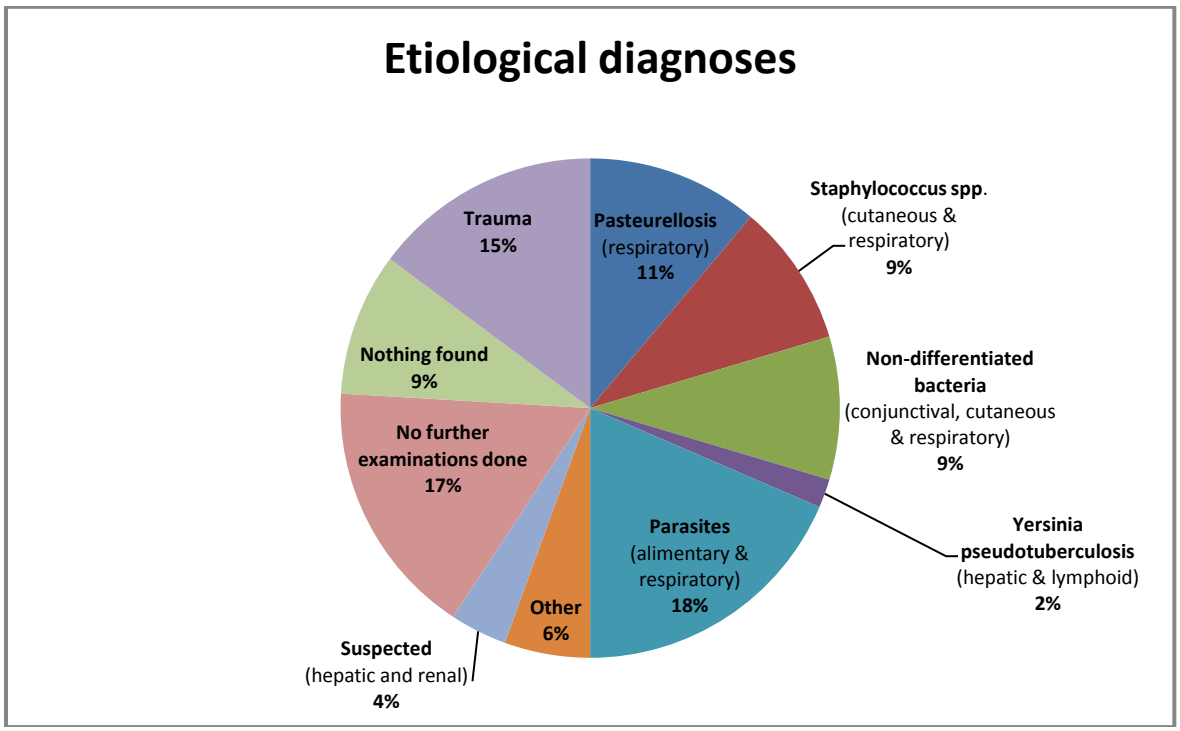


Figure 5 *The etiological and non-etiological diagnoses based on the findings or non-findings of diseases in 54 hares.*

The etiological diagnoses (59%) were mostly made in the respiratory system and overall, etiological agents were mostly bacteria. Etiological diagnoses included (broncho)pneumonic pasteurellosis; *Staphylococcus* dermatitis and pneumonia, of which the etiology was *Staphylococcus aureus* in 3 out of the 5 cases; (non-differentiated) bacterial conjunctivitis, dermatitis and bronchopneumonia; and hepatic and lymphatic yersiniosis. In the alimentary system, only parasitic etiological diagnoses were made, *Graphidium strigosum* gastritis (2%) and enteritic coccidiosis (11%). There was also a parasitic etiological diagnosis in the respiratory system, lungworm bronchopneumonia (≈6%). 'Other' etiological diagnoses were based on non-infectious diseases such as amyloidosis and extra-uterine pregnancy. There were two hares with a suspected etiological diagnosis, hepatic EBHS and *Encephalitozoon* nephritis. The non-etiological diagnoses, 'no further examinations done', 'nothing found' and 'trauma', were accounted for 41% (22 out of 54).

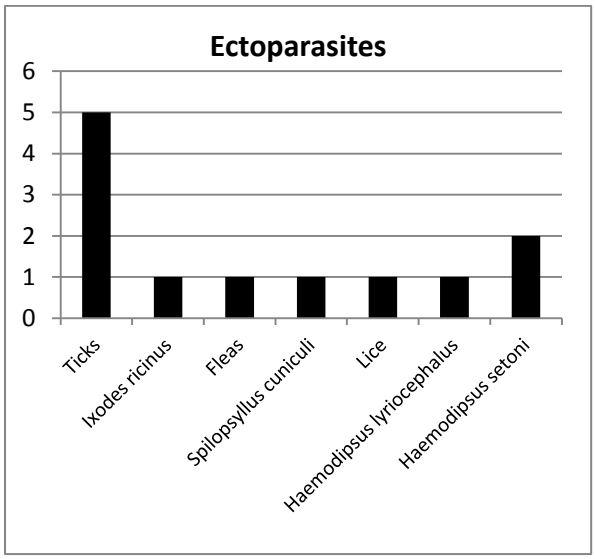


Figure 6 *The occurrence of ectooparasites in 54 hares.*

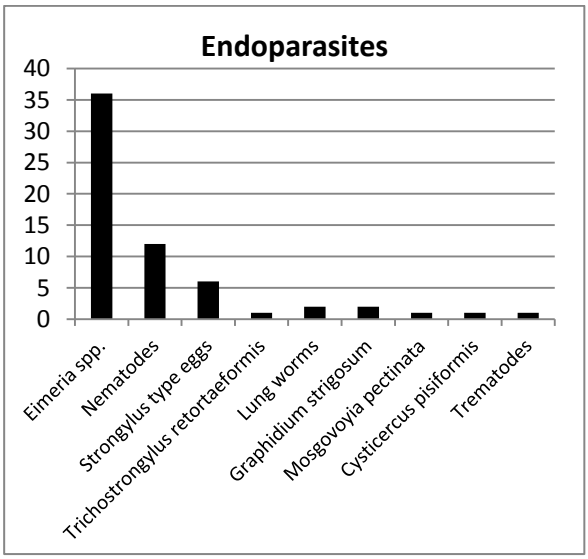
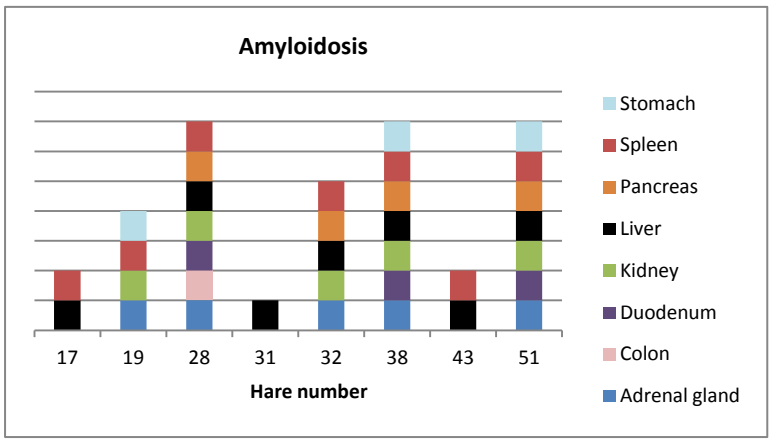


Figure 7 *The occurrence of endoparasites in 54 hares.*

As can be seen in figure 6, ticks were the ectoparasites which were found the most. Only one out of the six cases was differentiated as *Ixodes ricinus*. Fleas did not occur often, twice, of which one was determined as the rabbit flea *Spilopsyllus cuniculi*. The occurrence of lice, *Haemodipsus lyriocephalus* and *Haemodipsus setoni*, was also low.

The occurrence of endoparasites is shown in figure 7. *Eimeria* spp. was detected the most, in 36 cases, namely oocysts. Nematodes were found in twelve cases, with one case found in the lungs. *Strongylus* type eggs were found in faeces, *Trichostrongylus retortaeformis* in the lungs, and *Graphidium strigosum* in the stomach. Cestodes, *Mosgovoyia pectinata* and *cysticercus pisiformis*, were found in the alimentary tract and mesentery respectively.



The occurrence of amyloidosis is shown in figure 8. It has been detected in 8 out of 54 hares submitted. Amyloidosis was mostly found in the spleen and liver and least in the colon. Tularemia test results from the CVI were all negative.

Figure 8 *The occurrence of amyloidosis in different organs in 8 hares.*

The reproductive life cycle and its role in susceptibility to diseases

The most important phases in the reproductive life cycle of the European brown hare are shown in figure 9. In the Netherlands, female hares are pregnant during January up to and including September (Broekhuizen, Maaskamp 1981; Antoniou et al. 2008). This is not a steady period, as there is a peak of sexual behaviour in the spring (Flux, Angermann 1990). The phenomenon Mad March hares refers to the males gathering around one female in estrus, while the female tries to 'box off' the males with her forepaws. Males are polygamous and there is no bonding between male and female (Harcourt-Brown, Whitwell 2003). The European brown hare is a polyestrous seasonal breeder (Roellig et al. 2010) and thus can produce several litters a year, with an average of 3-5 litters a year (Flux, Angermann 1990). Listeriosis and brucellosis are mainly seen during pregnancy (Wuthe, Schönberg 1999; Treml et al. 2007). When the spring is cold, females tend to delay breeding and thus produce fewer litters in the breeding period. It also causes a decrease in litter size (Van Wieren et al. 2006). When the winter is mild, hares start breeding early and thus longer, with a higher percentage of pregnant females and higher litter sizes. But this can also increase incidence of diseases (Smith et al. 2005) due to higher hare densities and thus more disease transmission (Edwards et al. 2000).

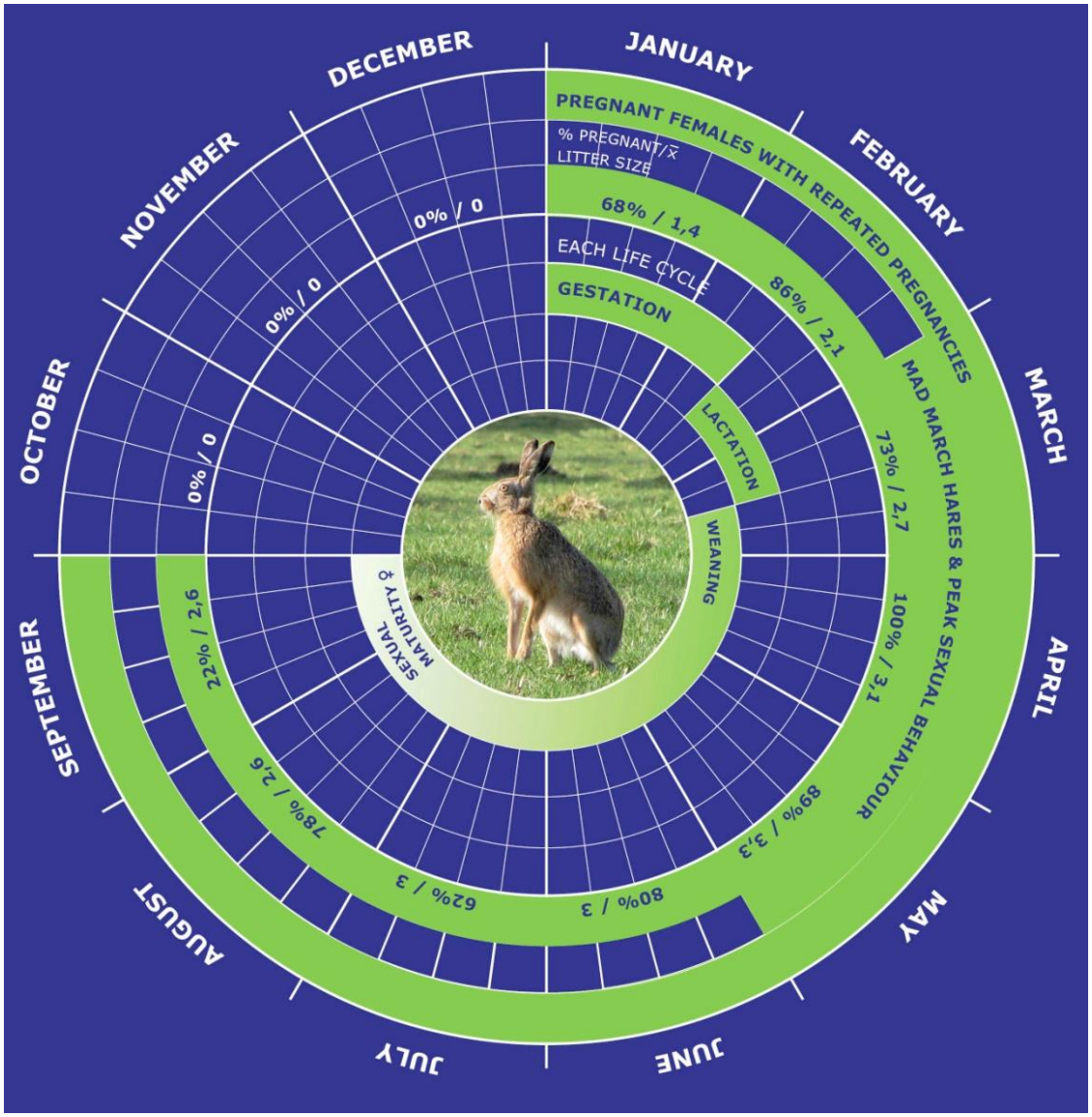


Figure 9 *The reproductive life cycle of the European brown hare (Broekhuizen, Maaskamp 1981; Harcourt-Brown, Whitwell 2003; Stott, Wight 2004; Stott, Harris 2006; Antoniou et al. 2008). Photography by Margriet Montizaan.*

The leverets, so called juveniles during lactation (Hacklander et al. 2002), are born in the open after 42 days of gestation (Flux, Angermann 1990; Harcourt-Brown, Whitwell 2003). At birth they are fully furred, open-eyed and have their running skills quickly under control, this is called being precocial (Harcourt-Brown, Whitwell 2003). Mothers leave their leverets after cleaning them. Leverets will also leave their place of birth, separately. They do return in the evening, shortly after sunset (Stott, Harris 2006), when the mother also returns for suckling (lactation) for 2-3 minutes (Flux, Angermann 1990; Harcourt-Brown, Whitwell 2003; Hacklander et al. 2002). Leverets are found to be highly susceptible to coccidiosis and lungworms (Frölich et al. 2003; Wibbelt, Fröhlich 2005). Gradually suckling decreases and leverets are weaned after 4 weeks. Then they are called juveniles (Harcourt-Brown, Whitwell 2003). Juveniles reach the adult size at 5 months, adults have an average weight of 3.8 kg (Flux, Angermann 1990). Rapidly, sexual maturity follows at approximately 6-7 months (Stott, Wight 2004). An adult disease is said to be EBHS. It does not occur under the age of 40 days (Wibbelt, Fröhlich 2005).

Protocol

A hare specific protocol has been created, as shown in Annex 4. It actually contains two protocols, a necropsy and a sampling protocol. These have been designed in regards to the most important diseases in the European brown hare. The necropsy protocol has been designed to simplify the process of writing down the necropsy and to serve as a reminder as each line is marked off and there is a categorized sequence. The standard protocol was used as a basis and therefore some items have remained the same. The sampling protocol has been designed to serve as a checklist for the necropsier and as a reminder for additional examinations.

Discussion

Pilot

The pilot was successful, because more cases were submitted from the four participating provinces compared to previous years. But the period before the pilot already showed an increase in cases compared to previous years. And also, higher results were found in provinces which did not participate in the pilot. This can either be explained by the fact that 2011 was a year in which more diseases occurred, or in which more people submitted hares. Anyhow, due to the positive results of the pilot, a broad national study could be considered in order to obtain more information about hare diseases in all of the Netherlands and their geographic patterns.

Hare reports

The most frequently found infectious diseases in the European brown hare in the Netherlands during 2008-2011 were pasteurellosis and coccidiosis. The most common morphological diagnosis found in this study was pneumonia.
Pasteurellosis in hares is caused by *Mannheimia hemolytica* and *Pasteurella multocida*. *P. multocida* is stated to be the most common, but *M. hemolytica* is said to cause the most infections in hares (Williams, Barker 2001). In this study, *P. multocida* was diagnosed twice and *M. hemolytica* once, but further conclusions cannot be drawn because the other three cases were not further determined. *M. hemolytica* is also said to cause purulent conjunctivitis (Devriese et al. 1991), but this was not found in this study. The same study (Devriese et al. 1991) stated that the conjunctivitis is also seen in hares with EBHS, but only one case was suspected of EBHS and it did not have conjunctivitis. Pasteurellosis in wild mammals causes pneumonia and septicaemia (Williams, Barker 2001). This was also the case in this study, as seromembranous morphological diagnoses, such as pericarditis and pleuritis were associated with *Pasteurella* spp. Coccidiosis can cause mortality in hares due to severe enteritis. It can also cause mesenteric lymphadenitis (Marcato et al. 1986). Next to the fact that the combination of the two was also seen in this study, *Eimeria* spp. were also seen in many hares with no enteritis. It is said that coccidiosis can also occur in healthy hares, and certain factors such as age and weather conditions can make hares more susceptible to the disease (Van Wieren et al. 2006). *Eimeria* spp. is said to affect juveniles the most (Wibbelt, Fröhlich 2005), but in this study this is not significantly found due to amount of reports with unknown age.
Although body condition is mostly poor in diseased hares, certain diseases can be seen in hares with good body condition due to (per)acute infection. Good body conditions have been reported in hares with toxoplasmosis (Sedlak et al. 2000) and tularemia (Wibbelt, Fröhlich 2005; Decors et al. 2011). Both diseases mostly occur in an acute form (Williams, Barker 2001). Neither was found in this study, although seven cases were noted as having a good body condition.
A virus which may occur in European brown hares is myxomatosis. This virus is mostly found in European wild rabbits, but can also transmit to hares when a great number of rabbits are infected (Wibbelt, Fröhlich 2005; Williams, Barker 2001). There is also a viral disease called fibromatosis. These two viral diseases are related and they give multiple nodules on the head (ears, eyelid and nose) and on the limbs (Wibbelt, Fröhlich 2005; Marcato et al. 1986). Although both viruses were not found in this study, one could suggest performing more research on these viruses if submitters keep on finding hares with thick and pussy eyes. *Staphylococcus* spp. were the second most found bacterial agents, with *Staphylococcus aureus* responsible for the greater part. Although it was primarily found in the cutaneous system, it could also be found in the respiratory and systemic system causing pneumonia, pericarditis, pleuritis and pericard fibrosis. This is more or less similar to described pathological findings in previous studies, as is seen in Annex 3. Pericard fibrosis is a pathological finding, which can be seen after a pericarditis has been survived (McGavin, Zachary 2007).

Hepatic etiological diagnoses seemed to be scarce. This is odd because the liver is one of the most affected organs by infectious diseases as can be seen in Annex 3. The liver can be affected by systemic diseases, such as yersiniosis or tularemia. Yersiniosis has been diagnosed once. It can be caused by two Gram negative rods, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. Since *Yersinia enterocolitica* is revealed to have similar clinical and pathological signs as *Y. pseudotuberculosis*, disease of both is referred to as yersiniosis. Pseudotuberculosis is an old reference to only *Yersinia pseudotuberculosis* (Frölich et al. 2003; Williams, Barker 2001). Yersiniosis can occur as an acute, subacute or chronic condition. Sometimes the spleen is also enlarged (Williams, Barker 2001). The liver can also be the main target, as is the case in EBHS. This is a highly contagious calicivirus, related to RHDV. It mainly causes acute necrotic hepatitis (Williams, Barker 2001; Fröhlich, Lavazza 2008). Periportal necrosis with little to none inflammatory reaction has been described as the primary microscopic lesion (Gavier-Widen 1994). The chronic form can also occur and showed chronic hepatitis, fibrosis and bile duct proliferation. This form has been diagnosed by demonstration of the EBHS antigen (Williams, Barker 2001; Gavier-Widen 1994). Liver fibrosis can result from hepatocellulair necrosis and can strongly affect a working liver (McGavin, Zachary 2007). In this study, only one case was suspected of EBHS, but more of these hepatic cases could fit the description. Encephalitozoonosis was the suspected disease in one case of nephritis. *Encephalitozoon* spp. are microsporidia. While *Encephalitozoon cuniculi* was more expected, because it is a well-known microsporidium in rabbits, the only found species in hares are *E. intestinalis* and *E. hellem*. (De Bosschere et al. 2007).

Questions can be raised on etiological diagnoses which were not further differentiated. For example, coccoid Gram positive bacteria could be either *Staphylococcus* spp. or *Streptococcus* spp. *Staphylococcus aureus* being the most likely one, looking at results. These bacteria can be cultured and identified. The same goes for *Pasteurella* spp. If these etiological diagnoses would have been more specified, more could have been said about the prevalence. In parasites it happened as well, these parasites could be determined by parasitology.

Ticks may serve as vectors in transmitting infectious diseases and zoonoses, such as tularemia. Hares can also serve as a reservoir for *Borrelia burgdorferi*, which can cause the disease of Lyme in humans. Ticks are known to infect humans with *B. burgdorferi* (Talleklint, Jaenson 1993). *Ixodes ricinus* is the most common tick in the Netherlands (Wielinga et al. 2006). *Spilopsyllus cuniculi*, the flea that was found in one case and probably in two cases, is said to be the main vector for myxomatosis, a virus occurring in wild European rabbits. As said before, hares may also be infected when their habitat is in the same area as rabbits (Wibbelt, Fröhlich 2005; Kenis, Roques 2010). Two kinds of lice occur in European brown hares, *Haemodipsus lyriocephalus* and *Haemodipsus setoni*. *H. setoni* was later on discovered to be different from *H. lyriocephalus*. Therefore, for a long time, *H. lyriocephalus* was thought to be the only louse occurring in the European brown hare in the Netherlands. *H. setoni* may occur more often, which was also seen in this study. Lice can be seen more in the spring and summer than in the other seasons. It is thought that due to infection, a diseased hare does not clean its skin as often at it would do in a healthy state. And so, it is more susceptible to lice. More infections with lice were seen in other diseases with a chronic character. Lice may also be a vector for tularemia (Broekhuizen 1971).

Endoparasites are often seen in wild animals, such as hares (Wibbelt, Fröhlich 2005). Nematodes are roundworms which occur in the respiratory tract and the alimentary tract of hares (Bowman, Lynn 1999). In European brown hares, *Protostrongylus* spp. occur in the lungs, *Graphidium strigosum* in the stomach, and *Trichostrongylus retortaeformis* and *Trichuris leporis* in the small intestines (Wibbelt, Fröhlich 2005; Bowman, Lynn 1999). In this study, not many endoparasites were found except for *Eimeria* spp. Lungworms were reported, referring to *Protostrongylus communatus* (or *P. pulmonalis*) or *P. tauricus* (Frölich et al. 2003; Soveri, Valtonen 1983). The low occurrence of lungworms in this study could be explained by its life cycle. Lungsworms have an indirect cycle with snails being the intermediate host. Thus, when hares do not come in contact with snails, which live in specific habitats, the occurrence will be low (Frölich et al. 2003). Subclinically, alimentary nematodes are mostly seen in adult hares (Wibbelt, Fröhlich 2005). In this study, *Strongylus* type eggs were seen,

which refers to a collective term for eggs of the *Strongyloidea*, *Trichostrongyloidea* and *Ancylostomatoidea* superfamilies (Bowman, Lynn 1999). As *Trichostrongylus retortaeformis* is the only nematode in hares in these families, most likely these eggs are of *Trichostrongylus retortaeformis*. *Trichuris leporis*, an endoparasite of the caecum (Wibbelt, Fröhlich 2005), was not found in this study. *Cysticercus pisiformis* is the bladderworm of *Taenia pisiformis*. The end host is the dog and the hare can become an intermediate host when faeces of the dog are ingested (Bowman, Lynn 1999). The reason for the low occurrence of *cysticercus pisiformis* in this study can be due to hygiene measures during hunting. Since the dogs no longer get to eat organs of possible infected hares, incidence has declined (Soveri, Valtonen 1983). *T. pisiformis* and *Mosgovoyia* (also known as *Cittotaenia*) *pectinata* are both cestodes. The hare is the end host of *Mosgovoyia pectinata* (Wibbelt, Fröhlich 2005).

The only case of a trematode detected, most likely *Fasciola hepatica* or *Dicrocoelium dendriticum*, could also be explained by the indirect life cycle and the fact that hares do not like places where cattle and sheep, the end host of these trematodes, are herded (Soveri, Valtonen 1983; Santilli, Galardi 2006; Taylor et al. 2007).

A remarkable diagnostic finding in this study was the relatively high occurrence of amyloidosis, although reports could be found in literature. Hares are likely to develop amyloidosis due to chronic infections. They can even die from secondary amyloidosis due to its damage to the kidneys and the liver (Harcourt-Brown, Whitwell 2003). Most predisponing organs include spleen, liver, kidneys and adrenal glands which have parenchymatous characters. Amyloid deposits may also be found in the alimentary tract. It is possible that in different species, different organs may be affected (Jakob 1971). Parasitic infections may be one of the primary causes, but idiopathic amyloidosis may occur as well (Geisel, Linke 1988). In this study, spleen and liver were found the most, kidney and adrenal gland being a good second, and last and least in alimentary tract, just as these previous findings have stated. Amyloidosis was seen in combination with all kinds of parasites, cestodes, nematodes and coccidiosis, but also with bacterial infections.

Some diseases did not occur in this study. Beginning with the disease one would want to see the least, tularemia. Tularemia is caused by the Gram negative rod *Francisella tularensis*. The agent is highly infectious, also for humans, and among others uses bloodsucking vectors (Williams, Barker 2001). Brucellosis in hares is caused by *Brucella suis biovar 2*, it is a Gram negative short rod. It is mostly found in hares which live in areas with free-ranging pigs and wild boars (Frölich et al. 2003). The occurrence of pigs and/or boars in the areas from the submitted hares is unknown, but it could be the reason for the negative results of the present study. The incidence of listeriosis is rare and difficult to detect (Davis et al. 1981). It is caused by *Listeria monocytogenes*, which is a Gram positive rod (Williams, Barker 2001). Toxoplasmosis, an acute fatal disease (Frölich et al. 2003), in hares has been described in several studies (Frölich et al. 2003; Sedlak et al. 2000; Jokelainen et al. 2011). *Toxoplasma gondii* is a parasite which mainly occurs in cats. Hares are intermediate hosts and get infected through oocysts in cat faeces (Frölich et al. 2003; Wibbelt, Fröhlich 2005). The lack of cats in the areas from submitted hares can explain negative findings in this study. The occurrence of cats in the participated areas has not been a part of this study. Both *Eimeria* spp. and *T. gondii* produce oocysts, but these can be easily separated during cytology (Bowman, Lynn 1999).

The reproductive life cycle and its role in susceptibility to diseases

The length of breeding season can play a significant role in the susceptibility to diseases and especially leverets are susceptible to diseases. Because of the long breeding season, hares of all life stages can come in contact with different seasonal weather conditions. Many studies have shown that weather conditions such as rainfall, total rainfall and periods of heavy rainfall (Van Wieren et al. 2006), and cold temperatures have a negative effect on hare populations (Van Wieren et al. 2006; Hacklander et al. 2002; Jennings et al. 2006).
Listeriosis has been said to cause abortion and nervous clinical signs in neonates (Lecuit 2007). Brucellosis spreads during reproduction, due to aborted fetuses (Wibbelt, Fröhlich 2005; Treml et al. 2007) and among others produces pathological signs that can be seen in the reproductive tract (Williams, Barker 2001). Leverets have a higher susceptibility to parasitic diseases like coccidiosis and lungworms, *Protostrongylus* spp., due to their lack of age immunity (Harcourt-Brown, Whitwell 2003). *Protostrongylus pulmonalis* can also be seen in adult hares, because of its chronic infection in young hares (Soveri, Valtonen 1983). As mentioned before, the infection with *Eimeria* spp. does not have to result in disease, it also occurs in healthy hares. *Eimeria* spp. favours wet weather conditions and so, it may occur more frequently after periods of rainfall, and can infect more hares, young and old (Harcourt-Brown, Whitwell 2003; Van Wieren et al. 2006). Leverets are especially vulnerable to rainfall and cold temperatures. Leverets have little help to maintain their temperature. They have the disadvantage of being born on the ground, out in the open, only being nursed in the evening and no huddling with siblings. But they have to be independent at a young age, and therefore they do develop a thermoregulation capacity, to increase their heat production, at an early age (Hacklander et al. 2002). Thermoregulation can be disrupted and consume too much energy due to rainfall and cold temperatures, causing hypothermia (Van Wieren et al. 2006). This will also result in higher susceptibility to coccidiosis (Smith et al. 2005), and bacterial diseases such as pseudotuberculosis and pasteurellosis, which mainly occur during the winter. Pseudotuberculosis favours wet weather conditions and cold temperatures, dry periods limits incidence of pasteurellosis (Smith et al. 2005; Wibbelt, Fröhlich 2005).
Research has also shown that greater litter sizes have negative effects on body weights at birth, and as there are more mouths to feed, each leveret also gets less milk (Hacklander et al. 2002). Litter size has been shown to be related to the weather, but not to habitat and nutrition (Jennings et al. 2006). When breeding season is maximal, the leveret production is high. This makes hares, in particular juveniles who no longer are getting their maternal antibodies, susceptible for EBHS. This virus can rapidly kill when population is highly dense, which is in the fall (Edwards et al. 2000; Fröhlich, Lavazza 2008).
Thus, leverets are very susceptible to diseases, but also weather conditions can favour diseases. Breeding in the winter is mostly unfavourable, but as can be seen in figure 9, when neonates survive, they will reach sexual maturity in the same year and thus may contribute to the population in the same year as they were born (Flux, Angermann 1990; Stott, Wight 2004; Hacklander et al. 2002). *Treponema* sp. can occur during breeding season as it is a sexually transmitted bacterium (Harcourt-Brown, Whitwell 2003). *Staphylococcus aureus* can be found on the skin of healthy hares. It is when lesions occur that the disease produces pathological signs (Wibbelt, Fröhlich 2005). Hares are not territorial, but fights can occur in food scarcity and also during boxing which is a part of reproductive behaviour, as previously described (Harcourt-Brown, Whitwell 2003). In the Netherlands, highest pregnancy rates were seen in April, with 100% and highest litter sizes were seen in May, as seen in figure 9 (Broekhuizen, Maaskamp 1981). This can be evidence that although breeding can occur in the winter with bad weather conditions and thus with higher susceptibility to disease, most hares in the Netherlands reproduce in the most favourable season, the spring. Worth noting is an extra-uterine pregnancy in the present study. It was seen in a hare which was necropsied in late October. This is in contradiction to literature used for figure 9, which results showed that there should not be any pregnant females during October in the Netherlands.

Protocol

The protocol was designed for further investigations based on previous studies. Both necropsy and sampling protocols have a checklist model unlike the standard protocol.
A short description of additions and changes will now be discussed.

Ectoparasites bag

Ectoparasites may leave the hare after it has died and prepared for submission. So therefore, to check for ectoparasites, it is also preferred to check the bag in which the hare has been submitted.

Age

The terms 'Juvenile' and 'Adult' have been used in the past for age indication, based on size, absence/presence of the thymus and lactation. Determination of age should be easy to use during necropsy, but should also be accurate. Literature study showed an age determination method, which has been used in several studies as an easy way of determining age (Marboutin, Peroux 1995; Soveri, Valtonen 1983).
The method is based on the ossification of the ulna and radius, which can be divided in nine stages.

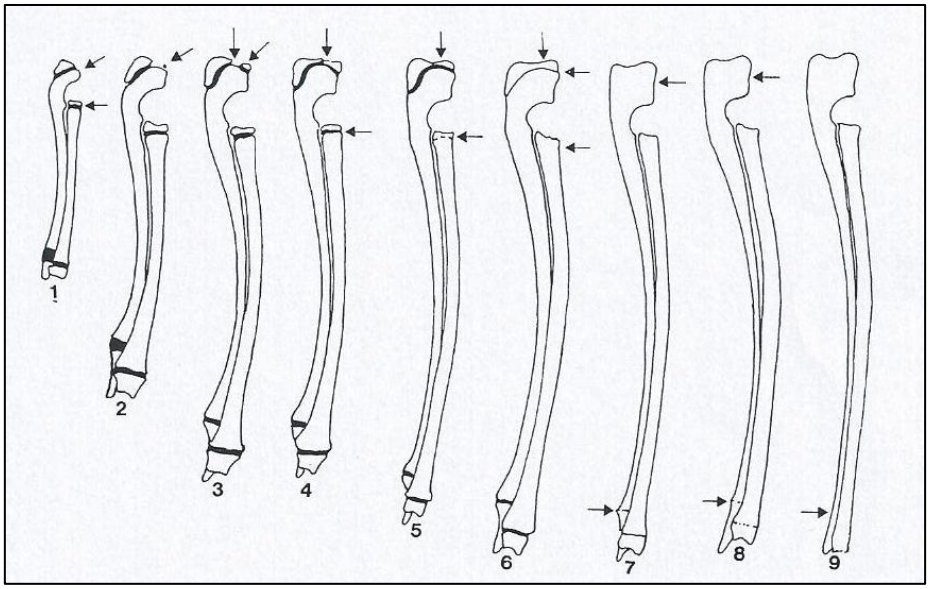


Figure 10 *A chart of the nine stages of ossification of the ulna and radius in the European brown hare (Broekhuizen, Maaskamp 1979).*

By means of palpation of distinguished marks, pointed out with arrows in figure 10, the stages and their age estimation can be determined. The chart gives an estimation up to seven to nine months which corresponds with stage eight or nine (Broekhuizen, Maaskamp 1979). Based on sexual maturity which is reached in six to seven months in most hares (Stott, Wight 2004), a proper classification of 'Juveniles' would be seven months and under, and 'Adults' would be older than seven months. Stages six to eight, corresponding to approximately five to eight months, would be the most important stages in order to distinguish between a juvenile and an adult. The method can also be used in cleaned bones, which can be a good practice method (Broekhuizen, Maaskamp 1979). It is not said to discard the standard indication characteristics, but to use this method as an addition, to improve age determination. Although, during the present and retrospective study, no fetuses or neonates were detected, these two age stages are included in the necropsy protocol in case of future detection.

Body condition

Body condition was a part of the standard protocol. But due to the lack of a specification, findings could be differently described. In order to use these findings for the analysis, they were categorized as described in the Materials and Methods. To generalize, a simple and hare specific body condition measurement was included in the created hare specific necropsy protocol. Studies have shown that

perirenal fat gives a good indication of the total amount of body fat. It is also said to be the largest fat deposit in hares (Bonino, Bustos 1998) and it reflects the medium-term body condition (Jennings et al. 2006). To simplify, the perirenal fat is categorized in: 'Good', 'Moderate' and 'Poor' (Gyuranecz et al. 2011). Subcutaneous fat is used in general in determining body condition, thus it serves as an extra tool.

Autolysis

Many hares that were seen during necropsy, already had some degree of autolysis. If autolysis is too severe, many organs cannot be used for examination. Therefore, to inform pathologists who were not present at the necropsy and readers of the reports, autolysis is added to the necropsy protocol.

Abscesses

Several infectious diseases in hares can cause abscesses, as can be seen in Annex 3. It can therefore help to make an etiological diagnosis and serve as a reminder to perform bacteriology, which can be found on the sampling protocol.

Diarrhea

As can be seen in Annex 3, diarrhea can occur in diseased hares with coccidiosis or yersiniosis (Williams, Barker 2001; Taylor et al. 2007). This clinical sign can be used in order to make an etiological diagnosis.

Bruising and (shot)wounds

In order to determine if external trauma was the cause of death, one could look for bruising and/or (shot)wounds. Usually this is already reported by the submitter, but this is not always the case and information from submitters is not always correct. Haemorrhagic clots can also be seen in external trauma, but these will be noted in the area where they have been seen.

Lnn (lymph nodes)

Not all lymph nodes are affected when a lymphadenitis occurs, as can be seen in Annex 3. Therefore, the main clusters of lymph nodes, which are mentioned in the necropsy protocol, should be separately examined. At necropsy, one could easily overlook a cluster. Therefore, it also serves as a reminder to check. Naturally, if an unmentioned cluster of lymph nodes shows pathological signs, it must also be noted.

Fluids

Fluids can be caused by autolysis, but also by certain diseases such as pasteurellosis. Hares with pasteurellosis may have serosanguinous or serogelatinous fluids in the thoracic cavity (Williams, Barker 2001). Fluids can be used for cytology and bacteriology and are therefore an item to be collected on the necropsy protocol.

Mesentery

The mesentery has a separate item, because of a specific parasite, *cysticercus pisiformis*. As mentioned before, this is the bladderworm of *Taenia pisiformis* and it can be seen with the naked eye as small oval cysts (Marcato et al. 1986).

Histopathology

Sampling for histopathology starts during necropsy. The samples are based on the standard protocol, with an addition of the eyes. Because there are many eye problems reported, it is useful to include the eyes in sampling. Because of their flaccid character, it should first be hardened by preservation before it can be used for histopathology. In order to do so, it can first be put in 4% buffered formaldehyde and later on be sampled for cassette fixation in paraffin. Other samples may be sampled the same way as previously done, one fitted-sized sample in the cassette, one extra sample in 4% buffered formaldehyde and finally, paraffin fixation.

Cytology

As said before, cytology can also be performed on fluids. Extra stains are separately mentioned, because they can be forgotten when extra staining occurs at the last minute.

-80°C

Sampling for future purposes was already done, but is now a part of the sampling protocol. It can be done during or shortly after necropsy.

Serology

Sera can be obtained from blood (clots) from the heart, then centrifuged, and if necessary stored at -20°C (Frölich et al. 2003). Serology can be used in detecting numerous diseases, as can be seen in Annex 3. The description can be used to inform about the amount of blood and for which disease, if possible to say, it could be used to detect.

Parasitology

The item about parasitology can be used as a checklist for further identification. Ectoparasites, preserved in 70% ethanol (Bowman, Lynn 1999; Taylor et al. 2007), should be collected for determination. Also stomach and small intestine mucosa samples could be used to identify parasites. Thus, these samples may be taken, kept in a bowl with a little water and then be identified by microscopy. The stomach parasite, *Graphidium strigosum*, may be seen on the stomach mucosa with the naked eye as small red worms (Taylor et al. 2007). *Trichostrongylus retortaeformis* could be sampled after *Strongylus* type eggs are detected. *Protostrongylus* spp., lungworms, can only be seen during histopathology. Possible 'other' places can also be sampled, if there is a suspicion or detection of parasites.

Tularemia

Since the occurrence of tularemia in several European countries, in both humans and hares, a monitoring has been set up by DWHC and CVI to identify tularemia in hares. Samples of the spleen and lung are sent to CVI and screened by PCR. CVI has a laboratory with a high level of containment (Anonymous 2011). The liver has also been used in studies to detect tularemia (Williams, Barker 2001; Müller et al. 2007; Runge, et al. 2011), which included identification by PCR (Runge et al. 2011). Till now, tularemia has not been identified in hares in the Netherlands.

Finally, it is worth noting that, during necropsy, it was seen that the heart is remarkably large. But this is a physiological condition and serves as an adaptation for the endurance of running in hares (Flux, Angermann 1990). Also, it is very important to take safety measures during necropsy, because of the occurrence of zoonoses in hares. These zoonoses can cause mild to severe infection in humans. The use of gloves is mandatory and is a safety measure to protect, for example, from pasteurellosis, which can be transferred to people by wound contamination (Williams, Barker 2001). More severe safety measures, on top of the protection with gloves, such as the use of a face mask for mucous membrane of the eyes and a mouth cap, should be taken for protection against tularemia. *Francisella tularensis* can cause clinical signs in very low doses (Treml et al. 2007), as little as 10 bacteria (Gyuranecz et al. 2010). During necropsy, intact and non-intact skin, mucous membranes and even inhalation can cause infection (Williams, Barker 2001).

In conclusion, this study showed that the number of hares submitted during the pilot have significantly contributed to the amount of examined and analyzed hares used in this study, although the results are still too limited to give a full analysis of the situation in the Netherlands. For the most part, bacterial and parasitic infectious agents were found, such as pasteurellosis, *Staphylococcus* spp., yersiniosis and coccidiosis. Because many cases in this study did not result in identification or differentiation of an etiological diagnosis, only an incomplete analysis of all the examined hares during 2008-2011 could be given. Therefore, further research should be more profound. The present study showed that there are differences in susceptibility to diseases due to the reproductive life cycle of hares. Especially leverets are more susceptible to diseases. From previous studies there were indications that weather conditions may also contribute to the susceptibility to diseases. The triangle relationship between reproductive life cycle, weather conditions and diseases should be further investigated. The created hare specific protocols for necropsy and sampling can be used for a more complete analysis in future studies. This study can be used as a starting point, a contribution to future studies on infectious diseases and their patterns in the European brown hare population in the Netherlands.

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Annex 1 *The standard protocol*

Necropsy number:
Species:

Exhibitor:
Contents cassette 1-5*:
Frozen storage**:
Bacteriology (only if indicated):

Cytology
HC*** liver:
HC spleen:
HC lung:
HC intestine:
Native intestine:
Stamp (only if indicated):
IFT: yes/no

Macroscopy

Necropsy date:
Pathologist/Specialist registrar:
Student:

Weight:
Age category:
Body condition:

Head & Neck:
Nose:
Ears/eyes:
Mouth cavity/teeth:
Tongue (including salivary gland):
Brain:
(Para) thyroid glands:

Thorax/respiration & circulation
In situ organs/ free fluid:
Trachea:
Pleura/diaphragm:
Lungs:
Heart & blood vessels:

Abdomen/ remaining internal organs
In situ organs/free fluid:
Stomach:
Duodenum/ pancreas:
Jejunum/ileum:
Colon:

Liver:
Spleen:
Lymph nodes:
Kidneys:
Adrenal glands:
Bladder:
Reproductive organ: m/f:

Skeleton/ limbs:
Mineralization:
Joints:
Food pads:

Temporary conclusion after macroscopy:

* Cassettes contain parts of standard organs: (para)thyroid glands, heart, spleen, liver, stomach, duodenum+pancreas, lungs, kidney, gonad, all deviations. Small parts of these organs are put in a cassette, which is put in 4% buffered formaldehyde when full. Together with this cassette, separate parts of the organs are put in the same 4% buffered formaldehyde. The cassettes are processed in paraffin blocks and are used for histopathologic examination.
** This is according to DWHC protocol, see annex 2.
*** HC is HemaColor staining

Annex 2 *An overview of hare reports during the period of 11/2/2008-31/12/2012*

	General information									Morphological diagnosis				
	Number	Date	Province	M/V/U	J/A/U	Kg	Submitted as		Body condition	Organ	Degree	Progress	Distribution	Type exsudate/others
1	B0800166	11-2-2008	Friesland	M	U	U	Found dead		Poor	Pericarditis	Severe	Chronic-acute areas	Multifocal	Fibrinous-fibrosis
1	B0800166	11-2-2008	Friesland	M	U	U	Found dead		Poor	Pleuritis	Severe	Chronic-acute areas	Multifocal	Fibrinous-fibrosis
1	B0800166	11-2-2008	Friesland	M	U	U	Found dead		Poor	Pneumonia	Severe	Subacute	Multifocal	Pyogranulomatous
2	B0800390	29-4-2008	Friesland	M	U	U	Euthanasia	Blunt trauma	Poor	Encephalitis	Severe	Subacute	Multifocal	Lymphoplasmacellular, necrosis
2	B0800390	29-4-2008	Friesland	M	U	U	Euthanasia	Blunt trauma	Poor	Liver fibrosis	Severe	Chronic	Multifocal	Biliar proliferation
2	B0800390	29-4-2008	Friesland	M	U	U	Euthanasia	Blunt trauma	Poor	Trauma				
3	3090116024	16-1-2009	Zeeland	M			Found dead (autolysis)							
4	3090715034	15-7-2009	Gelderland	V	U	U	Found dead		Poor	Jejuno-enteritis	Severe	Chronic	Multifocal	Necrotic
4	3090715034	15-7-2009	Gelderland	V	U	U	Found dead		Poor	Lymphadenitis	Severe	Chronic	Focal	Necropurulent
4	3090715034	15-7-2009	Gelderland	V	U	U	Found dead		Poor	Trauma				
5	3091019002	19-10-2009	Noord-Brabant	M	J	U	Euthanasia	Blunt trauma	Moderate	Bronchopneumonia	Severe	Subacute	Multifocal	Necropurulent
5	3091019002	19-10-2009	Noord-Brabant	M	J	U	Euthanasia	Blunt trauma	Moderate	Trauma				
6	3091022035	22-10-2009	Utrecht	M	U	3,4	Found dead		Poor	Trauma				
7	3091112036	12-11-2009	Zeeland	M	U	U	Euthanasia	Dog	Poor	Dermatitis	Severe	Chronic	Focal intensive	lymphoplasmacellular, necropurulent
7	3091112036	12-11-2009	Zeeland	M	U	U	Euthanasia	Dog	Poor	Trauma				
8	3091201031	1-12-2009	Zuid-Holland	M	U	U	Euthanasia	Shot	Poor	Conjunctivitis; CA				
8	3091201031	1-12-2009	Zuid-Holland	M	U	U	Euthanasia	Shot	Poor	Trauma				
9	3091201032	1-12-2009	Zuid-Holland	V	U	U	Euthanasia	Blunt trauma	Moderate	Conjunctivitis/blepharitis (CA)	Mild	Subacute	Diffuse	Necropurulent
9	3091201032	1-12-2009	Zuid-Holland	V	U	U	Euthanasia	Blunt trauma	Moderate	Trauma				
10	3091201033	1-12-2009	Zuid-Holland	M	U	U	Euthanasia	Shot	Poor	Conjunctivitis	Severe	Chronic	Bilateral	Mucopurulent
10	3091201033	1-12-2009	Zuid-Holland	M	U	U	Euthanasia	Shot	Poor	Trauma				
11	3091215063	15-12-2009	Friesland	M	U	U	Euthanasia	Dog	Good	Pneumonia	Severe	Subacute	Multifocal	Haemorrhagic, purulent
12	3091221015	21-12-2009	Zeeland	M	U	U	Euthanasia	Shot	Moderate	Bronchopneumonia	Severe	Chronic	Multifocal	Necropurulent
12	3091221015	21-12-2009	Zeeland	M	U	U	Euthanasia	Shot	Moderate	Trauma				
13	3100111017	11-1-2010	Friesland	M	U	2,2	Found dead		Moderate	Bronchopneumonia	Severe	Acute	Focal	Purulent
13	3100111017	11-1-2010	Friesland	M	U	2,2	Found dead		Moderate	Dermatitis	Moderate	Chronic	Multifocal	Purulent, ulcerative
14	3100702002	2-7-2010	Drenthe	V	U	1,3	Blunt trauma		Moderate	Trauma				
15	3100903050	3-9-2010	Zuid-Holland	U	U	U	Euthanasia	Shot	Cachectic	Ophtalmitis	Severe	Acute	Diffuse	Fibrinopurulent
15	3100903050	3-9-2010	Zuid-Holland	U	U	U	Euthanasia	Shot	Cachectic	Trauma				
16	3101013043	13-10-2010	Zuid-Holland	V	J	3	Found dead		Cachectic	Presence of coccidiosis				
17	3101021001	21-10-2010	Zuid-Holland	V	U	4,7	Found dead		Cachectic	Endometritis	Severe	Subacute	Diffuse	Necropurulent, pyogranulomatous
17	3101021001	21-10-2010	Zuid-Holland	V	U	4,7	Found dead		Cachectic	Enteritis	Mild	Subacute	Multifocal	Lymphoplasmacellular, purulent
17	3101021001	21-10-2010	Zuid-Holland	V	U	4,7	Found dead		Cachectic	Peritonitis	Severe	Chronic	Diffuse	
18	3101027004	21-10-2010	Zuid-Holland	V	A	3,4	Found dead		Moderate	Bronchopneumonia	Severe	Chronic	Multifocal	Granulomatous
18	3101027004	21-10-2010	Zuid-Holland	V	A	3,4	Found dead		Moderate	Tracheitis	Moderate	Subacute	Diffuse	Mucopurulent
19	3101028018	28-10-2010	Overijssel	M	U	3	Found dead		Cachectic	Gastritis	Moderate	Chronic	Multifocal	Mucous
20	3101111004	11-11-2010	Zuid-Holland	V	U	3,7	Euthanasia	Shot	Poor	Trauma				
20	3101111004	11-11-2010	Zuid-Holland	V	U	3,7	Euthanasia	Shot	Poor	Cataract				
21	3101207008	7-12-2010	Gelderland	V	U	3,7	Euthanasia	Shot	Good	Enteritis	Mild	Subacute	Multifocal	Plasmahistiocytair, purulent
21	3101207008	7-12-2010	Gelderland	V	U	3,7	Euthanasia	Shot	Good	Trauma				
22	3101207009	7-12-2010	Gelderland	V	U	2,3	Euthanasia	Shot	Good	Trauma				
23	3101230016	30-12-2010	Zuid-Holland	V	J	2,7	Unknown		Cachectic	Bronchopneumonia	Severe	Subacute	Diffuse	Granulomatous
24	3101230017	30-12-2010	Zuid-Holland	U	U	2,1	Unknown		Cachectic	Bronchopneumonia	Severe	Chronic	Diffuse	Fibrinopurulent
24	3101230017	30-12-2010	Zuid-Holland	U	U	2,1	Unknown		Cachectic	Pericarditis	Little	Chronic	Focal	Lymphoplasmacellular
25	3110111004	11-1-2011	Gelderland	M	U	3,7	Blunt trauma		Moderate	Trauma				
26	3110113003	13-1-2011	Zuid-Holland	M	U	2,2	Found dead		Poor	Nephritis	Moderate	Acute	Bilateral, tubulo-interstitial	Lymphoplasmacellular
27	3110204008	4-2-2011	Drenthe	V	U	3,2	Euthanasia	Blunt trauma	Cachectic	Presence bacteria adrenal gland				
27	3110204008	4-2-2011	Drenthe	V	U	3,2	Euthanasia	Blunt trauma	Cachectic	Hepatitis	Severe	Chronic	Multifocal	Pyogranulomatous
27	3110204008	4-2-2011	Drenthe	V	U	3,2	Euthanasia	Blunt trauma	Cachectic	Lymphadenitis	Severe	Chronic	Multifocal	Pyogranulomatous
27	3110204008	4-2-2011	Drenthe	V	U	3,2	Euthanasia	Blunt trauma	Cachectic	Splenitis	Severe	Chronic	Multifocal	Pyogranulomatous
27	3110204008	4-2-2011	Drenthe	V	U	3,2	Euthanasia	Blunt trauma	Cachectic	Trauma				
28	3110225023	25-2-2011	Friesland	M	A	2,5	Extern trauma		Poor	Nephritis	Severe	Chronic	Multifocal, interstitial	Lymphoplasmacellular
28	3110225023	25-2-2011	Friesland	M	A	2,5	Extern trauma		Poor	Trauma				
29	3110520049	20-5-2011	Drenthe	V	A	4	Euthanasia	Shot	Moderate	Trauma				
30	3110615041	15-6-2011	Zuid-Holland	V	A	1,5	Unknown (used as bait)							
31	3110701009	1-7-2011	Overijssel	V	A	U	Found dead		Cachectic	Bronchopneumonia	Mild	Acute	Multifocal	Fibrinopurulent, seropurulent
32	3110727002	27-7-2011	Overijssel	M	U	2,9	Found dead		Cachectic	Pleuritis	Moderate	Acute	Diffuse	Fibrinopurulent
32	3110727002	27-7-2011	Overijssel	M	U	2,9	Found dead		Cachectic	Pneumonia	Severe	Chronic	Focal	Necropurulent
32	3110727002	27-7-2011	Overijssel	M	U	2,9	Found dead		Cachectic	Pericarditis	Moderate	Acute	Diffuse	Fibrinopurulent
33	3110831011	31-8-2011	Noord-Brabant	V	U	3	Euthanasia	Blunt trauma	Poor	Endometritis	Severe	Subacute	Diffuse	Necropurulent
33	3110831011	31-8-2011	Noord-Brabant	V	U	3	Euthanasia	Blunt trauma	Poor	Pneumonia			Multifocal	
33	3110831011	31-8-2011	Noord-Brabant	V	U	3	Euthanasia	Blunt trauma	Poor	Trauma				
34	3110901031	1-9-2011	Zeeland	V	U	2,5	Found dead		Cachectic	Hepatitis	Mild	Subacute	Multifocal, random	Necropurulent
35	3110912003	12-9-2011	Utrecht	M	J	2	Found dead		Cachectic	Presence of coccidiosis				
36	3110912005	12-9-2011	Zuid-Holland	V	A	3,1	Found dead		Cachectic	Hepatocellular necrosis	Severe	Acute	Multifocal/centrolobular	Eosinophil inclusion bodies
37	3110916025	16-9-2011	Groningen	M	U	1,4	Found dead		Poor	Enteritis	Mild	Acute	Diffuse	Haemorrhagic
38	3110928046	29-9-2011	Zuid-Holland	M	U	2,9	Found dead		Good	Presence of amyloidosis				
39	3111010003	10-10-2011	Gelderland	V	A	2,6	Found dead		Cachectic	Enteritis		Subacute	Diffuse	Purulent
39	3111010003	10-10-2011	Gelderland	V	A	2,6	Found dead		Cachectic	Lymphadenitis		Subacute	Multifocal	Necropurulent
40	3111011002	11-10-2011	Zuid-Holland	V	A	2,2	Found dead		Cachectic	Enteritis	Moderate		Diffuse	
41	3111026006	26-10-2011	Limburg	V	U	2,6	Found dead		Cachectic	Lymphadenitis		Chronic	Multifocal	Necropurulent
42	3111101003	1-11-2011	Utrecht	V	J	1,9	Found dead		Cachectic	Enteritis	Little	Subacute	Focal	Purulent
43	3111107055	7-11-2011	Drenthe	V	A	2,8	Euthanasia	Dog	Poor	Bronchopneumonia	Severe	Acute	Multifocal	Purulent
43	3111107055	7-11-2011	Drenthe	V	A	2,8	Euthanasia	Dog	Poor	Trauma				
43	3111107055	7-11-2011	Drenthe	V	A	2,8	Euthanasia	Dog	Poor	Dermatitis	Moderate	Chronic	Focal	Necrotic
44	3111108028	8-11-2011	Zuid-Holland	M	A	3,7	Euthanasia	Shot	Good	Dermatitis	Severe	Chronic	Focal	Granulomatous, necropurulent
44	3111108028	8-11-2011	Zuid-Holland	M	A	3,7	Euthanasia	Shot	Good	Pericard fibrosis	Little	Chronic	Multifocal	
44	3111108028	8-11-2011	Zuid-Holland	M	A	3,7	Euthanasia	Shot	Good	Pneumonia	Moderate	Acute	Multifocal	Fibrinopurulent
44	3111108028	8-11-2011	Zuid-Holland	M	A	3,7	Euthanasia	Shot	Good	Trauma				
44	3111108028	8-11-2011	Zuid-Holland	M	A	3,7	Euthanasia	Shot	Good	Periportal liver fibrosis				Biliar proliferation
45	3111115001	15-11-2011	Limburg	V	U	3	Euthanasia	Shot	Good	Enteritis	Mild-moderate	Chronic	Diffuse	Lymphoplasmacellular
45	3111115001	15-11-2011	Limburg	V	U	3	Euthanasia	Shot	Good	Periportal liver fibrosis	Moderate			
45	3111115001	15-11-2011	Limburg	V	U	3	Euthanasia	Shot	Good	Trauma				
46	3111122035	22-11-2011	Zuid-Holland	V	U	2	Found dead		Cachectic	Hepatitis	Mild	Chronic	Pericholangial	Lymphoplasmacellular, necropurulent
47	3111122037	22-11-2011	Zuid-Holland	V	U	2,5	Found dead		Cachectic	Pneumonia	Mild	Chronic	Diffuse, interstitial	Necropurulent
47	3111122037	22-11-2011	Zuid-Holland	V	U	2,5	Found dead		Cachectic	Periportal liver fibrosis	Mild	Acute	Pericholangial	Lymphoplasmacellular, biliar proliferation
47	3111122037	22-11-2011	Zuid-Holland	V	U	2,5	Found dead		Cachectic	Hepatocellular necrosis		Acute	Diffuse	
48	3111122038	22-11-2011	Zuid-Holland	M	U	2,3	Found dead		Cachectic	Peritonitis	Severe	Acute	Diffuse	Fibrinous
48	3111122038	22-11-2011	Zuid-Holland	M	U	2,3	Found dead		Cachectic	Periportal liver fibrosis	Mild		Pericholangial	Biliar proliferation
48	3111122038	22-11-2011	Zuid-Holland	M	U	2,3	Found dead		Cachectic	Hepatocellular necrosis	Mild			Necropurulent
48	3111122038	22-11-2011	Zuid-Holland	M	U	2,3	Found dead		Cachectic	Pneumonia	Mild	Chronic	Diffuse, interstitial	Necropurulent
49	3111123002	23-11-2011	Drenthe	M	A	3,2	Euthanasia	Dog	Cachectic	Trauma				
49	3111123002	23-11-2011	Drenthe	M	A	3,2	Euthanasia	Dog	Cachectic	Dermatitis	Severe	Chronic	Multifocal	Necropurulent, ulcerative
49	3111123002	23-11-2011	Drenthe	M	A	3,2	Euthanasia	Dog	Cachectic	Pneumonia	Mild	Chronic	Diffuse, interstitial	Necropurulent
49	3111123002	23-11-2011	Drenthe	M	A	3,2	Euthanasia	Dog	Cachectic	Lymfohyperplasia				
50	3111129010	29-11-2011	Gelderland	M	U	3,5	Euthanasia	Shot	Cachectic	Trauma				
50	3111129010	29-11-2011	Gelderland	M	U	3,5	Euthanasia	Shot	Cachectic	Pneumonia	Mild	Chronic	Diffuse, interstitial	Necropurulent
51	3111201001	1-12-2011	Utrecht	V	A	3	Found dead		Cachectic	Pneumonia	Mild	Chronic	Diffuse, interstitial	Necropurulent
51	3111201001	1-12-2011	Utrecht	V	A	3	Found dead		Cachectic	Pneumonia	Moderate	Acute	Multifocal	Purulent
51	3111201001	1-12-2011	Utrecht	V	A	3	Found dead		Cachectic	Nephritis	Moderate	Chronic	Multifocal, interstitial	Lymphoplasmacellular, necropurulent
52	3111206001	6-12-2011	Overijssel	V	A	3	Found dead		Moderate	Dermatitis	Moderate	Subacute	Multifocal	Lymphoplasmacellular, necropurulent, necrosis
52	3111206001	6-12-2011	Overijssel	V	A	3	Found dead		Moderate	Hepatitis	Mild	Subacute	Multifocal	Necropurulent
52	3111206001	6-12-2011	Overijssel	V	A	3	Found dead		Moderate	Pleuritis	Mild	Acute	Diffuse	Fibrinous
52	3111206001	6-12-2011	Overijssel	V	A	3	Found dead		Moderate	Pneumonia	Severe	Acute	Multifocal	Necropurulent
53	3111216002	16-12-2011	Zeeland	V	A	3,3	Euthanasia	Shot	Poor	Trauma				
53	3111216002	16-12-2011	Zeeland	V	A	3,3	Euthanasia	Shot	Poor	Dermatitis	Severe	Chronic	Multifocal	Lymphoplasmacellular, necropurulent, ulcerative
53	3111216002	16-12-2011	Zeeland	V	A	3,3	Euthanasia	Shot	Poor	Ophtalmitis	Severe	Chronic	Diffuse	Fibrinopurulent
53	3111216002	16-12-2011	Zeeland	V	A	3,3	Euthanasia	Shot	Poor	Lymfohyperplasia				
54	3111216003	16-12-2011	Zeeland	V	A	3,4	Euthanasia	Shot	Cachectic	Trauma				
54	3111216003	16-12-2011	Zeeland	V	A	3,4	Euthanasia	Shot	Cachectic	Dermatitis	Moderate	Chronic	Multifocal	Necropurulent
55	3111229042	29-12-2011	Overijssel	M	A	2,5	Found dead		Cachectic	Presence of coccidiosis	Mild			
56	3111229043	29-12-2011	Overijssel	M	A	2,9	Euthanasia	Shot	Good	Trauma				

	Ectoparasites	Endoparasites	Other	Negative results	Etiological diagnosis
1		*Eimeria* spp. oocysts, *Strongylus* type eggs			*Pasteurella* sp. & *Pseudomonas luteola* (lung)
2	Fleas, ticks	*Eimeria* spp. oocysts		Ziehl-Neelsen; ABC *Listeria* ; *Toxoplasma*	Nothing found
3					
4		*Eimeria* spp. oocysts		IFT	*Eimeria* spp. (intestine)
5		Worm larvae, *Strongylus* type egg, *Eimeria* spp. oocysts		Ziehl-Neelsen	*Pasteurella* sp. (lung)
6		Nematoda (duodenum/colon), *Eimeria* spp. oocysts	LV dilatation, RV hypertrophy		Trauma
7		*Eimeria* spp. oocysts			*Bordetella* spp. (dermis)
8		*Eimeria* spp.			Trauma
9		*Eimeria* spp. oocysts, *Strongylus* type eggs		PAS	Cocoid Gram + bacteria (eyelid)
10		*Eimeria* spp.			*Enterobacter* spp. & *Citrobacter* spp. (swab eye)
11					*Pasteurella* sp. (lung)
12					Gram-negative rods (non-fermenter) (lung)
13		Nematoda (duodenum), *Eimeria* spp. oocysts (small intestine)			*Mannheimia hemolytica* (lung)
14		*Eimeria* spp., trematoda eggs			Trauma
15			Coccoid bacteria (eye)	Silver stain	Coccoid bacteria (eye)
16	Tick	*Eimeria* spp.	RV hypertrophy		Nothing found
17		*Eimeria* spp. oocysts	Amyloidosis (liver, spleen)		Extra-uterine pregnancy; amyloidosis
18	Ticks	*Eimeria* spp. oocysts, lungworms & larvae		PCR Herpes; *Francisella* (no indicator)	Lungworms (lung); Nothing found (tracheitis)
19	*Haemodipsus setoni*	Graphidium strigosum (stomach), *Eimeria* spp. oocysts	Amyloidosis (adrenal glands, kidneys, spleen, stomach)		*Graphidium strigosum* ; amyloidosis
20		*Eimeria* spp. oocysts, *Clostridium spiroforme* , nematoda			Trauma
21		*Eimeria* spp., nematoda larvae (duodenum)			*Eimeria* spp. (intestine)
22					Trauma
23		*Strongylu*s type eggs, lungworms & larvae, *Eimeria* spp.			Lungworms (lung)
24		*Eimeria* spp., nematoda (lung)		Ziehl-Neelsen	Lungworms (lung)
25					Trauma
26				Ziehl-Neelsen; Gram stain	Suspected of Encephalitozoon (kidney)
27					*Yersinia pseudotuberculosis* (liver, spleen, lymph nodes)
28		*Cysticercus pisiformis* (mesentery)	Amyloidosis (adrenal glands, colon, kidney glomeruli, liver, pancreas, small intestine, spleen)		Amyloidosis; *cysticercus pisiformis* ; unknown (kidney)
29		Nematoda (duodenum)			Trauma
30					
31	Ticks	*Mosgovoyia pectinata* (alimentary tract),*Eimeria* spp. oocysts	Amyloidosis (liver)	Ziehl-Neelsen, Tularemia	*Pasteurella multocida* (lung)
32	Ticks	*Eimeria* spp. oocysts, *Strongylus* type eggs	Amyloidosis (adrenal glands, kidneys, liver, spleen)	Ziehl-Neelsen, IFT, Tularemia	*Staphylococcus aureus* (lung)
33		*Eimeria* spp. oocysts		Tularemia	No further examinations done
34	*Spilopsyllus cuniculi*	*Trichostrongylus retortaeformis* (duodenum), *Graphidium strigosum*		Ziehl-Neelsen, Tularemia	No further examinations done
35		*Eimeria* spp.		Tularemia	Nothing found
36				VHD, Tularemia	Suspected of EBHS (liver)
37		*Eimeria* spp.	*E.coli* (lung)	Salmonella, Yersinia, Tularemia	*Eimeria* spp. (intestine)
38	*Ixodes ricinus*	*Eimeria* spp. oocysts	Amyloidosis (adrenal glands, duodenum, kidney, liver, pancreas, spleen, stomach)	Tularemia	Systemic amyloidosis, no indicator
39		*Eimeria* spp. oocysts		Tularemia	*Eimeria* spp. (intestine, galbladder)
40		*Eimeria* spp. oocysts (also biliar)		Tularemia	*Eimeria* spp. (intestine, galbladder)
41		*Eimeria* spp. oocysts, nematoda (duodenum)		Ziehl-Neelsen, Tularemia	No further examinations done
42		*Eimeria* spp. oocysts, nematoda (duodenum)		Tularemia	*Eimeria* spp. (intestine)
43		*Eimeria* spp. oocysts, nematoda (duodenum)	Amyloidosis (liver, spleen)	Ziehl-Neelsen, Tularemia	*Pasteurella multocida* (lung)
44		*Eimeria* spp. oocysts, nematoda (duodenum)		Tularemia	Streptococci and Staphylococci (abscess dermis)
45		*Strongylus* type eggs, budding yeast		*Salmonella* , *Clostridium perfringens* or coliforms, Tularemia	Nothing found
46				Tularemia	No further examinations done
47				Tularemia	No further examinations done
48				Tularemia	No further examinations done
49		*Eimeria* spp. oocysts, nematoda (duodenum)	Coccoid bacteria (nose)	Bacteria (lung), Tularemia	*Staphylococcus aureus* (abscess)
50	*Haemondipsus setoni* & *H. lyriocephalus*	*Eimeria* spp. oocysts		Tularemia	No further examinations done
51		*Eimeria* spp. oocysts	Amyloidosis (adrenal glands, kidney, liver, pancreas, spleen)	Ziehl-Neelsen, Tularemia	*Staphylococcus pseudointermedius* (abscess); amyloidosis
52	Lice	*Eimeria* spp. oocysts, nematoda (duodenum)		Whartin-Starry, Tularemia	No further examinations done
53				Tularemia	*Staphylococcus aureus* (dermis)
54				Tularemia	No further examinations done, possible same as 53
55		*Eimeria* spp.		Tularemia	Nothing found
56		*Eimeria* spp.		Tularemia	Trauma

Annex 3 *Most important differential diagnoses in European brown hares*

		Disease/parasite	Pathological findings	Diagnostics
Skin	*Bacteria*	Pasteurellosis*	Necropurulent abscesses	Wright/Giemsa stain
		Staphylococcus aureus*	Necropurulent abscesses	Gram stain/Culture
		Treponema spp.	Purulent dermatitis (oral & prepuce)	Bosma-Steiner stain/Serology
		Yersiniosis*	Diarrhea	Fecal culture
	Parasites	Coccidiosis	Diarrhea	Cytology
		Haemodipsus spp.	Alopecia, spots	Microscopy
		Ixodes spp.	Spots	Microscopy
		Spylopsyllus spp.	Spots	Microscopy
Eye	*Bacteria*	Pasteurellosis*	Purulent conjunctivitis	Culture/PCR
		Staphylococcus aureus*	Conjunctivitis	Gram stain/Culture
Nose	*Bacteria*	Pasteurellosis*	Rhinitis (necrotic, hemorrhagic/fibrinopurulent)	Culture/PCR
Cardiovascular	*Bacteria*	Pasteurellosis*	Serofibrinous pericarditis (necrotic,hemorrhagic/fibrinopurulent)	Culture/PCR
		Staphylococcus aureus*	Endocarditis, pericarditis	Gram stain/Culture
Respiratory	*Bacteria*	Pasteurellosis	Fibrinopurulent bronchopneumonia (necrotic/hemorrhagic), fibrinohemorrhagic interstitial pneumonia, fibrinous pleuritis	Culture/PCR
		Staphylococcus aureus	Necropurulent abscesses	Gram stain/Culture
		Tularemia*	Fibrinous pneumonia/pleuritis	Gram stain/IHC/Culture/PCR
		Yersiniosis*	Multifocal pyogranulomatous noduli, serofibrinous pneumonia	Culture
	Parasites	Protostrongylus spp.	Granulomatous bronchopneumonia	Histopathology/Microscopy
Liver	*Viruses*	EBHS	Severe acute periportal necrosis (necrotic hepatitis)	IHC/ELISA/PCR/serology
	Bacteria	Brucellosis	Necropurulent granulomatous noduli	Culture/PCR/Serology
		Listeriosis*	Miliary necrotic foci	Gram stain/Culture/IHC
		Tularemia*	Multifocal necrosis	Gram stain/IHC/Culture/PCR
		Yersiniosis*	Multifocal pyogranulomatous noduli	Culture
	Parasites	Coccidiosis (E.stiedai)	White noduli, bile duct proliferation	Cytology/Fecal culture/Flotation
		Fasciola hepatica*	Necrosis, pyogranulomatous inflammation, fibrosis	Parasitology/ELISA
		Toxoplasmosis*	Multifocal miliary necrosis	Antigen detection/ IHC
Spleen	*Bacteria*	Brucellosis	Necropurulent granulomatous noduli	Culture/PCR/Serology
		Listeriosis*	Focal necrotic foci	Gram stain/Culture/IHC
		Tularemia*	Multifocal necrosis	Gram stain/IHC/Culture/PCR
		Yersiniosis*	Multifocal pyogranulomatous noduli	Culture
Kidney	*Viruses*	EBHS	Tubular cell necrosis	IHC/ELISA/PCR/serology
	Bacteria	Staphylococcus aureus*	Necropurulent abscesses	Gram stain/Culture
	Parasites	Encephalitozoonosis	Focal interstitial nephritis	Gram/Giemsa stain/Histopathology/ELISA
Alimentary	*Bacteria*	Yersiniosis*	Mild-severe necrotic gastro-enteritis	Culture
	Parasites	Coccidiosis	Catarrhal/hemorrhagic enteritis, diffuse noduli	Cytology/Faecal culture/Flotation
		Graphidium strigosum	Ulceration (stomach)	Macroscopy/Microscopy/Sporulation
		Mosgovoyia pectinata	Catarrhal enteritis (small intestine)	Macroscopy/Microscopy
		Trichostrongylus retortaeformis	Catarrhal enteritis (small intestine)	Cytology/ Fecal culture/Histopathology/Sporulation
		Trichuris leporis (caecum)	Necrosis	Histopathology/Parasitology
Mesentery	*Parasites*	Cysticercus pisiformis	Cysts	Microscopy
Peritoneum	*Bacteria*	Pasteurellosis*	Peritonitis	Culture/PCR
		Staphylococcus aureus*	Peritonitis	Gram stain/Culture
		Yersiniosis*	Peritonitis	Culture
Lymph nodes	*Bacteria*	Staphylococcus aureus*	Purulent/necropurulent (superficial)	Gram stain/Culture
		Toxoplasmosis*	Necrosis	Antigen detection / IHC
		Tularemia*	Multifocal pyogranulomatous foci (abdomen)	Gram stain/IHC/Culture/PCR
		Yersiniosis*	Mesenteric lymphadenitis	Culture
	Parasites	Coccidiosis	Mesenteric lymphadenitis	Cytology/Histopathology
Brain	*Bacteria*	Listeriosis*	Purulent meingoencephalitis	Gram stain/Culture/IHC
	Parasites	Encephalitozoonosis	Focal granulomatous, pseudocysts encephalitis	Gram/Giemsa stain/Histopathology/ELISA
Bone marrow	*Bacteria*	Tularemia*	Multifocal necrosis	Gram stain/IHC/Culture/PCR
Reproductive tract	*Bacteria*	Brucellosis	Necropurulent granulomatous orchitis/ endometritis	Culture/PCR/Serology
		Listeriosis*	Abortion: necropurulent placentitis	Gram stain/Culture/IHC
		Pasteurellosis*	Pyometra, orchitis (subacute/chronic)	Culture/PCR
		Staphylococcus aureus*	Metritis	Gram stain/Culture

* **Zoonosis**

An overview of the most important differential diagnoses occurring in the European brown hare, based on literature (Davis et al. 1981; Soveri, Valtonen 1983; Marcato et al. 1986; Devriese et al. 1991; Lumeij et al. 1994; Von Damoser, Hofer 1995; Lumeij 1996; Williams, Barker 2001; Frölich et al. 2003; Taylor et al. 2007; Ziege et al. 2009; Jokelainen et al. 2011).

Annex 4 *The European brown hare necropsy and sampling protocol*

European brown hare (*Lepus europaeus*) necropsy protocol

Number: ……. Date: ……

Submitter: ……

Ectoparasites bag ☐ Absent ☐ Present, description: ……

Sex ☐ Male ☐ Female ☐ Unknown

Weight …… kg
Age ☐ Fetus ☐ Neonate ☐ Juvenile ☐ Adult

Body condition:
Perirenal fat ☐ Poor ☐ Moderate ☐ Good

Subcutaneous fat ☐ Poor ☐ Moderate ☐ Good

Autolysis ☐ Mild ☐ Moderate ☐ Severe

Photography ☐ No ☐ Yes, of……

Extern (skin/fur total body)
Ectoparasites ☐ Absent ☐ Present, description……

Fractures ☐ Absent ☐ Present, description……
Lesions, (shot)wounds:
Head/neck ☐ Absent ☐ Present, description……

Thorax/abdomen ☐ Absent ☐ Present, description……

Perineum/genitalia ☐ Absent ☐ Present, description……

Abscesses ☐ Absent ☐ Present, description……

Diarrhea ☐ Absent ☐ Present, description……

Intern (skin/subcutaneous total body)
Bruising ☐ Absent ☐ Present, description……

Cervical (superficial) lnn Description……

Joints/mineralization Description……

Head/neck
Eyes Description……

Nose Description……

Mouth Description……

Brain Description……

Thorax
In situ Description……

Pleural cavity Description……

Fluid ☐ Absent ☐ Present, description……

Trachea Description……

Lung Description……

Mediastinal lnn Description……

Pericardial fluid ☐ Absent ☐ Present, description……

Heart Description……

Vessels Description……

Abdomen

In situ Description……

Stomach Description……

☐ Parasites , description……

Duodenum+pancreas Description……

Caecum Description……

Colon Description……

Mesentery ☐ Parasites, description……

Mesenteric lnn Description……

Liver Description……

Spleen Description……

Kidney Description……

Adrenal glands Description……

Bladder Description……

Genital tract Description……

European brown hare (*Lepus europaeus*) sampling protocol

Histopathology (in formalin & cassette 1-5)

☐ Brain ☐ Liver ☐ Duodenum + pancreas

☐ Bone marrow ☐ Lung ☐ Lymph nodes, description……..

☐ Eye ☐ Kidney ☐ Adrenals

☐ Gonad ☐ Spleen ☐ Other, description……..

☐ Heart ☐ Stomach ☐ Other, description……..

Cytology

Liver ☐ No details ☐ Description……..

Spleen ☐ No details ☐ Description……..

Lung ☐ No details ☐ Description……..

Faeces ☐ No details ☐ Description……..

Native faeces ☐ No details ☐ Description……..

☐ Fluids, description……

☐ Extra stains Description……

Result:……..

-80

☐ Brain ☐ Kidney ☐ Bone marrow

☐ Heart ☐ Spleen ☐ Gonad

☐ Lung ☐ Stomach ☐ Blood

☐ Liver ☐ Intestines ☐ Other, description……..

☐ **Serology** Description……

☐ **Bacteriology** Description……

Parasitology

☐ Ectoparasites ☐ Stomach ☐ Small intestine ☐ Other, description……
……

Tularemia

☐ Spleen ☐ Lung