Pathological evaluation and comparison of the hare (*Lepus europaeus*)
population of the German north-sea island Pellworm and European mainland

Diploma thesis

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## List of abbreviations

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<tr>
<td>ca</td>
<td>Circa</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>°C</td>
<td>Degrees centigrade</td>
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<tr>
<td>DWD</td>
<td>Deutscher Wetterdienst</td>
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<td>EBHS</td>
<td>European Brown Hare Syndrome</td>
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<td>EBHSV</td>
<td>European Brown Hare Syndrome Virus</td>
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<td>e.g</td>
<td>Exempli gratia</td>
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<td>F</td>
<td>Female</td>
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<td>Fig.</td>
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<td>g</td>
<td>Gramm</td>
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<td>H&amp;E stain</td>
<td>Haematoxylin and eosin stain</td>
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<td>i.e</td>
<td>Id est</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
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<td>IUCN</td>
<td>International Union for Conservation of Nature and Natural resources</td>
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<tr>
<td>km</td>
<td>Kilometers</td>
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<tr>
<td>Km/h</td>
<td>Kilometers per hour</td>
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<td>km²</td>
<td>Square kilometer</td>
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<td>m</td>
<td>Meter</td>
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M  Male
mg  Milligram
PCR  Polymerase chain reaction
spp.  Species pluralis
sp.  Species
Tab.  Table
µm  Micrometer
1. Introduction

1.1 Study aim

Over the past years (1955/56-2006) great variations of the European brown hare (*Lepus europaeus*) population have been detected in the German province Schleswig-Holstein based on hunting bags (SKÍRNISSON, 1990; BORKENHAGEN, 2011). Similar variations were also recognized in other provinces in Germany (BORKENHAGEN, 2011). In fact a general decline of the European brown hare population has been observed in whole Europe since the 1960s (PETROV, 1976; CHAPMAN and FLUX, 1990; MITCHELL-JONES, 1999; SMITH et al., 2005).

On the red list for endangered species from the International Union for Conservation of Nature and Natural resources (IUCN) the European brown hare is ranked as “least concern” (SMITH and JOHNSTON, 2008). But as described in REICHLIN (2006) the European brown hare is on several national Red Lists of Threatened Species classified as “near threatened” or even “threatened” e.g. in Austria, Germany, Norway and Switzerland (REICHLIN et al., 2006).

On the German north-sea island Pellworm a decrease of the population was detected by local hunters in the years 2007 and 2008. In 2006 the hunting bag was 600 individuals; in 2007 it had decreased to 300 and in 2008 to 200 animals (pers. comm. EWERS). Based on this development a health screening program was started to find reasons for this development. The aim of this health screening was to evaluate the health status of the local European brown hare population and to find possible reasons to explain the decreasing population trend.

Present diploma thesis describes the pathohistological changes and compares them to diseases found on the mainland in other populations, as part of a complex project.
1.2 Study area

The 36.2 km² large North Frisian, marsh island Pellworm (54°,31’N,8°38’E) is located on the North Sea coast of Germany and belongs to the German federal state of Schleswig–Holstein. Hares were introduced on the island in 1856 -1870 by the Danish dike inspector Muhl and since then the hare population steadily increased. The introduced hares were all from the German province Schleswig-Holstein (BORKENHAGEN, 2011). Today the island is inhabited by the European brown hare along with the European wood mouse (*Apodemus sylvaticus*), the brown Norway rat (*Rattus norvegicus*), the house mouse (*Mus musculus*), the field mouse (*Microtus arvalis*), the hedgehog (*Erinaceus europaeus*), the water vole (*Arvicola amphibious*) and the muskrat (*Ondatra zibethicus*). Predator mammals are absent, except some feral free-range house cats (BORKENHAGEN, 2011). During the breeding season the island is also inhabited by a few birds of prey, such as the Marsh Harrier (*Circus aeruginosus*) and the Montagu’s Harrier (*Circus pygargus*) (HOFMANN, 2003).
2. Literature survey

2.1 European Brown Hare (*Lepus europaeus*)

The European brown hare belongs to the class mammalia and to the order lagomorphs. The order is represented by the family *Leporidae* and *Ochotonidae*. Twelve genera and 45 species are associated with the family *Leporidae* (HUTCHINS, 2003).

At first hares were integrated in the order rodents, but since there were just a few superficial similarities with the rodents and fossil remains showed that rodents and lagomorphs have different origins, it was suggested that they should form an own order in 1972 (ANGERMANN and THENIUS, 1972). As suggested, the hares nowadays form the order lagomorpha (HUTCHINS, 2003).

The European brown hare has a body size of about 52 - 68cm and a weight around 2.500 - 6.000g (HUTCHINS, 2003). Many characteristics of the European brown hare like long legs, long feet, large hearts, dark muscles with a lot of myoglobin, wide airway passage for breathing and a very light built skeleton, are all physical adaptations to be able to run fast (CHAPMAN and FLUX, 1990). The coat color remains brown the whole year (HUTCHINS, 2003).

Today the European brown hare is present almost all over Europe, from Iran in the south to western Siberia in the north. Because of great importance as game species, they have also been introduced to new areas like Ireland, Scandinavia, Chile, Argentina, North America, south Siberia, Australia and New Zealand (CHAPMAN and FLUX, 1990; HUTCHINS, 2003). Even though they can be found on farmland, moorland, open woodlands and steppes, they prefer to live in open landscapes, where they have a chance to escape their enemies due to their high speed. They can reach a top speed of 70 km/h (ZÖRNER, 1996).

The diet of the European brown hare is strict herbivore and consists mainly of grass such as *Bromus mollis*, *Triticum aestivum*, *Secale cereale*, *Festuca rubra*, *Lolium perenne*, *Poa annua*, *Poa pratensis*, *Arrhenaterum elatius* and *Phleum pretense* (BORKENHAGEN, 2011). The large parts of the plants are hard to digest, therefore
they have developed a specialized dietary adaption in form of a giant caecum, which contains ten times the volume of their stomach (HUTCHINS, 2003). It is assumed that the microflora of the caecum is adapted to boost digestion of the plants as seen in many other herbivore species (HERDT, 2002).

The hare is a caecotroph species and the produced caecotrophe contains almost four to five times more vitamins than normal feces and coprophagy is therefore seen as an essential behavior, making it easier for the hare to survive longer periods of limited food availability and unfavorable weather conditions (HUTCHINS, 2003).

It is a fact that the European brown hare is very sensitive to natural enemies as well as changes in weather conditions. Thus it is important that the reproduction behavior can replace the main losses during a year, to avoid declines of the population. One solution is the duration of the breeding season, which lasts almost the whole year, from January until October. In the remaining period the reproduction organs are in a state of rest, and the gonad-tissue loses weight as well as size.

Females reach sexual maturity at the age of eight months and males at the age of six months. The gestation period is about 42 - 43 days (SCHNEIDER, 1978). Females give birth to one-two newborns at first birth, but the amount of leverets can increase with the number of births. The mean value is described to be one-four newborns per litter and two-four litters per year (ANGERMANN and THENIUS, 1972). To increase the reproduction output even more, the female hare is capable of superfetation. Superfetation describes the fact that female hares are fertile for a further litter before the present one is born (RÖLLIG et al., 2010). The well-developed newborns of the European brown hare have fur from the time of birth, open eyes and are able to move right after birth. The weight at birth is 90 - 150g, depending on the size of the litter (ANGERMANN and THENIUS, 1972).

The newborns are suckled once a day for a period of roughly 33 days, with a very nutritious milk (SCHNEIDER, 1978). Although the energy content of the milk varies depending on the phase of reproduction and seasons of the year it is a very nutritious milk with a fat content of around 20% (ZÖRNER, 1996; VALENCAK and RUF, 2009). Through its lack of social contact the hare is a prey hard to find for their enemies (HUTCHINS, 2003).
All these qualities make the European brown hare well adapted to its habitat and the surrounding environment.

2.2 Causes of decline

According to PEGEL (1986) there are three main reasons for the decline in a hare population. At first the mortality rate is directly influenced by predators, hunting, traffic, poisoning and diseases. Secondly factors like environmental influences, the climate and availability of food. And thirdly, factors with an influence on the reproductive success.

ZÖRNER (1996) describes the four main reasons for the decline in a population as: diseases, enemies, climate/location/habitat and human influences. The intensification of agriculture and the changes in habitat as a result are also described as an important reason for a declining population (SMITH et al., 2005).

There are many pathogens causing diseases in hares. In the following paragraphs only the most common parasites, bacteria and viruses and those found in the present study are mentioned.

2.2.1 Parasites

Protozoa are eukaryotic, mono-cellular organisms. In the group protozoa the coccidia are a part of the class sporozoa. Coccidia, especially *Eimeria* spp., are one of the most important protozoa causing diseases in wild animals (Tab.1). Most of these parasites affect the gastrointestinal tract of the host animal, just a few types the epithelial and endothelial layers of the kidney and liver (NICKEL, 1987).

The development cycle of the parasites consists of three phases. The first phase is the asexual reproduction, schizogony. During this phase the sporozoites form schizonts which contain merozoites. These merozoites can infect enterocytes. In the second phase named gametogony, the merozoites form gametes. These then divide into micro- und macrogametes. Microgametes fertilize the macrogamets and zygotes are formed. The maturation of the spores, where zygotes transform into oocysts, is also called sporogony and embodies the third phase. The sporogony takes place...
outside the host and during this period the parasite is very sensible against weather conditions (NICKEL, 1987; MCGAVIN and ZACHARY, 2009).

The most common symptoms of a coccidian infestation are indigestion and watery-mucous diarrhea. Other common symptoms include emaciation, pale mucosa, weight-loss, muscle cramps and slow movements with a bent back (KÖTSCHE and GOTTSCHALK, 1990). In a severely affected intestine it is possible to see the infestation with *Eimeria* macroscopically. The intestine is then covered with up to millet seed-sized white spots (HACKLÄNDER et al., 2006).

Among wild animals the hare and the wild rabbit (*Oryctolagus cuniculus*) are often infested with coccidia (NICKEL, 1987). This is due to the coprophagy and the fact that all *Eimeria*-species are highly host- and tissue specific (HACKLÄNDER et al., 2006; MCGAVIN and ZACHARY, 2009).

Encephalitozoonosis is caused by the intracellular developing microspore - *Encephalitozoon cuniculi*. A chronic often latent disease, that occurs worldwide as an enzootic disease among rabbits (KÖTSCHE and GOTTSCHALK, 1990). In hares *Encephalitozoon* (*E. intestinalis* and *E. hellem*) have been described as well (DE BOSSCHERE et al., 2007).

The microspora shows a close relationship to fungi and is a very small, monocellular, obligatory parasite (DEPLAZES et al., 2013). The infestation rate among captive breeding rabbits in Europe, without any symptoms of the disease, is about 8–16%, whereas among rabbits with neurological symptoms it is about 85% (DEPLAZES et al., 2013). In the wild hare population just a few cases are reported (KÖTSCHE and GOTTSCHALK, 1990). The parasite is found in the central nervous system, spleen, salivary gland, kidney, liver, macrophages and in the urine. The most common pathologic findings of diseased rabbits are multifocal, necrotizing to granulomatous encephalitis together with a chronic, non-purulent, interstitial nephritis (MCGAVIN and ZACHARY, 2009).

The tapeworms, *Cestoda* spp., are a part of the group Platyhelminthes. The worms are dorsoventrally flattened, have a segmented body, are built in a link chain
Cestoda infestations among the hares are not seen as often as in the rabbit population (BOCH and SCHNEIDAWIND, 1988). The most common way for hares to get infested is through an oral uptake of the very common intermediate host, the moss mite (*Oribatida* sp.).

Mild infestations do often progress without or with minimal symptoms. A heavy infestation causes disorders especially in subadults, e.g. diarrhea, but can also lead to death through exhaustion (NICKEL, 1987). The *Leporidae* are both cyst- and tapeworm carriers. The adult living tapeworms in the hare mostly belong to the family *Anoplocephalidae* and rarely to the family *Davaineidae* (KÖTSCHE and GOTTSCHALK, 1990).

The hare is an intermediate host for *Taenia pisiformis*, the tapeworm of the dog. An infection takes place through oral uptake of eggs or proglottids, with the food. Hatched gubs migrate from the gastrointestinal tract in the bloodstream and develop into *Cysticercus pisiformis* in the liver (KÖTSCHE and GOTTSCHALK, 1990). *Taenia pisiformis* is seen as a pea-sized cyst, aggregated in grape-formed masses on the omentum, mesenterium, peritoneum and under the serosa of liver, stomach, spleen, lungs and other organs. The body migration through the liver causes “migrations-tracts” and due to following reparation the liver gets a firmer consistence (NICKEL, 1987). In infested hares and wild-rabbits a morphological specific hepatitis is often seen (DEPLAZES et al., 2013). Larger quantities of cysts in the intermediate hosts can cause peritonitis with consecutive death (NICKEL, 1987).

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*. Definitive hosts are members of the family *Felidae*. Some domestic animals, many wild animals, including the hare and also humans act as intermediate hosts (NICKEL, 1987). Among wild animals hares together with winged game species are affected more commonly. Some authors assume that toxoplasma infestations are the cause for as
many as 80% of the spontaneous deaths in the hare population (NICKEL, 1987). Others do not rank toxoplasmosis as a disease of big importance. They hypothesize that although there can be a high rate of infestation (30-50%) in a population it will, normally, just lead to single losses (KÖTSCHKE and GOTTSCALK, 1990). DEPLAZES et al. (2013) report a seroprevalence of 2.6% in Europe. Affected hares are inactive with a reduced appetite. Other symptoms like diarrhea and respiratory problems are also frequently seen (NICKEL, 1987).

The most common post mortem findings are an enlargement of spleen, intestinal lymph nodes and liver. The liver is pale and fragile with a few petechiae. The lungs can be edematous and swollen, the airways red and filled with foamy liquids (NICKEL, 1987).

2.2.2 Bacteria

Tularemia, also known as “Rabbit fever”, is caused by the bacterium, *Francisella tularensis*. The disease is known in Europe since it was found in Sweden in 1930 (SCHRÖDER, 1987). *Francisella tularensis* is a very contagious agent and is listed as a class A biothreat agent, as one out of just ten listed bacteria possible to affect both animals and human (TWENHAUFEL et al., 2009).

In case of an acute form of the disease, the spleen is swollen and an enlargement of lymph nodes is seen. In the chronic form emaciation together with granulomatous hepatitis and caseating necrosis of lymph nodes can be observed. In hares tularemia often presents itself as a hemorrhagic sepsis and the affected hare dies within 4-12 days. Tularemia has zoonotic potential and hunters are an endangered group (KÖTSCHKE and GOTTSCALK, 1990).

Primary pathologic findings which are macroscopically noticeable are 0.1-0.5 cm, grey-white foci and nodules in some or numerous organs in almost every chronic case of tularemia in diseased hares. These could therefore act as a warning sign for humans with contact to hares. The European brown hare is an important reservoir of this bacterium (GYURANECZ et al., 2010).
Brucellosis, a further zoonotic disease, with a plague like spreading, caused by a bacterium from the genus *Brucella*, was first found in 1887 by David Bruce (SCHRÖDER, 1987). The most common way for hares to get infected is grazing where swine abortions through *Brucella suis* have taken place (KÖTSCHE and GOTTTSCHALK, 1990).

Two forms of Brucellosis are known, acute and chronic. It is assumed that these two forms also appear among the free-ranging hares. The acute form is not seen often due to the hyperacute death of affected hares. The chronic form is more common. The hares demonstrate a few nonspecific symptoms like emaciation with less vitality and are therefore often shot, hit by cars or taken by predators (KÖTSCHE and GOTTTSCHALK, 1990). Common changes seen in a male hare is an enlargement of the testes together with a swollen and discolored penis. In female hares the reproductive organs are also affected, causing mostly suppurative vaginitis and vulvitis, the ovaries can be affected as well.

*Escherichia coli* is a part of the bacterial class *Escherichia* and belong to the family *Enterobacteriaceae*. *Escherichia coli* is a part of the normal intestinal flora from the large intestine in rabbits (WHITNEY, 1976). If there is a disorder leading to a change in the intestinal bacteria balance, the amount can increase. This is regularly seen in feeding defects or problems with feed hygiene (KÖTSCHE and GOTTTSCHALK, 1990). The importance of an *Escherichia coli* infection in wild animals is not entirely clarified. According to the great amount of several serotypes disease can occur, either as a sepsis or an intestinal infection (SCHRÖDER, 1987).

Pasteurellosis also called hemorrhagic septicaemia is caused by *Pasteurella multocida*. In the hyperacute form diseased individuals die within twelve to 48 hours, in the more rarely occurring subacute form it takes days to weeks (BOCH and SCHNEIDAWIND, 1988; KÖTSCHE and GOTTTSCHALK, 1990). The pathological changes seen in the acute form are hemorrhagic laryngotracheitis, petechiae and a considerable enlargement of liver and spleen (BOCH and SCHNEIDAWIND, 1988; KÖTSCHE and GOTTTSCHALK, 1990).
The bacteria can stay infectious up to months in cadaver, feces or soil (BOCH and SCHNEIDAWIND, 1988). Lack of food, wet- and cold weather and parasite infestations are supporting factors for Pasteurellosis (KÖTSCHE and GOTTSCHALK, 1990). If the above described factors appear, it can lead to an enzootic spreading of the disease and to high losses in the hare population. Up to 80% of the population can succumb to the disease. However, as soon as the weather gets dry and sunny, the epidemic state will decrease. It is presumed that Pasteurella sp. adapt very well to the actual host. This means when there is an enzootic spreading in an animal population, e.g. hares, it will mostly only affect hares. Poultry game and hoofed games will usually not get infected in the same spreading and vice versa (FRÖLICH et al., 1997).

2.2.3 Viruses

European brown hare syndrome (EBHS) is a disease specific for the European brown hare. It is caused by a Calicivirus and was discovered in Sweden 1980 (GAVIER-WIDÉN and MÖRNER, 1991).

The most common symptom of the disease is an acute, necrotizing hepatitis. EBHS occurs only in areas where European brown hares are found, therefore areas without the European brown hare are free from EBHS disease (LENGHAUS et al., 2000). The virus cannot affect hares younger than 50 days. Different studies have shown that populations in areas where the EBHS-prevalence is proven to be very high do not decline, as presumable after an outbreak. Instead they are stable and the mortality rate is low. It is presumed that less pathogenic lines are circulating in free-ranging populations (SCICILUNA et al., 1994; LENGHAUS et al., 2000; FRÖLICH et al., 2003).

2.3 Environmental factors

Weather dependent reasons are another important reason for huge numbers of losses in the group of the subadults. Long winters with crusted snow, resulting in long
periods of food shortage, is especially hard for subadult individuals already affected by parasites or other chronic diseases. In cold and wet summers there can be high losses of subadults. Researches in Denmark have found a connection between the temperature in the months of March to June, the rainfall in the months of June and July and the population density (KÖTSCHE and GOTTSCHALK, 1990). Further studies show the same result - that a high amount of precipitation, especially in the end of March and the beginning of April, has a significant negative influence of the net growth rate of the population (HOFFMANN, 2003). Other facts that have great influence on the hare population are predators, e.g. the red fox (*Vulpes vulpes*) (PEGEL, 1986). The importance of the fox has been proven through comparing mainland data with the islands Föhr and Pellworm where no predation occurs (BORKENHAGEN, 2011).
3. Material and methods

52 hares (22 males, 30 females) were collected during a hunt in October 2011 on the German north-sea island, Pellworm. The hunt was performed during the normal hunting season in cooperation with local hunters and the Research Institute of Wildlife Ecology, Vetmeduni Vienna, as part of a health screening of the hare population on Pellworm. As soon as possible after death the hares were brought to a local hunting lodge were necropsy was performed.

Age estimation was done using the sign of “Stroh” (STROH, 1931) and the lens weight (SUCHENTRUNK et al., 1991). Palpating the sign of “Stroh” means looking for the epiphyseal cartilage plate of the lower extremity and feeling the ossification of the epiphysis plate of the ulna/the epiphyseal protrusion of the ulna. For the age estimation through the lens weight, one eye is removed and fixed in 10 % formalin. The fixed lens is prepared, washed, dried at 100° Celsius for 24-26 hours and then weighed (measured in milligram). Hares up to one year, subadults, have a lens weight beneath 270 milligram. Older hares show an age-dependent, but higher weight (PEGEL, 1986) (Fig.1).

The body weight of the hares was measured with a digital scale, in grams (g), straight after death (Tab. 2 and 3).

For all hares a protocol was assigned at the time of necropsy and they all were sequentially numbered with one to 52. The protocol and the assigned number followed the hare throughout the whole survey. All macroscopical changes were written down in these protocols, and the findings were gathered and evaluated.

Tissue samples of all organs were taken and fixed in 7% neutrally buffered formalin. For pathohistological examination, all collected samples were embedded in paraffin wax, cut at 3µm, mounted on glass slides and stained with haematoxylin and eosin (H&E) according to standard procedures.

Further special staining methods were performed when deemed necessary: Congo-Red-staining, Ziehl-Neelsen, modified Ziehl-Neelsen, Gram, Giemsa and also a
Polymerase Chain Reaction (PCR) for detecting acid-fast bacteria. Ziehl-Neelsen is used to stain acid-fast, rod bacteria. Mycobacterium is an acid-fast bacteria and appears red due to staining with carbolic fuchsine and alcoholic hydrochloric acid (BAUMGÄRTNER et al., 2012).

Congo-Red is a special stain-method used to stain amyloid. Amyloid comprises pathological insoluble protein fibrils; they arise from improperly folded proteins and polypeptides, occurring naturally in the body. Amyloid is found around reticular and collagenous filaments in different organs in the body, chiefly in the kidney and the liver (BOSS, 1993; BAUMGÄRTNER et al., 2012).

In two cases immunohistochemistry (IHC) was performed to confirm the presence of *Encephalitozoon cuniculi*. Immunohistochemistry is used to detect specific chemical connections between peptides and proteins through an antigen-antibody reaction. The slides with an unknown antigen are incubated in a solution, containing antibodies against the assumed antigen. If the slides contain the correct antigen, the antibodies will bind to them. To make them visible, the antibodies are marked (WELSCH, 2010). The slides were evaluated using a microscope type Olympus BX51TF (Olympus corporation Tokyo, Japan).

The detected pathological and pathohistological changes were gathered in an Excel file for descriptive statistical analyzes. Statistical significance was calculated using Excel as described in VEJDE (2013).
4. Results

From 52 collected hares 30 were female and 22 male. 35 were evaluated as adult and 17 as subadult due to classification by using the sign of Stroh and the lens-weight. The 30 female hares could be divided into seven subadults and 23 adults and the 22 male hares into ten subadults and twelve adults (Fig.1).

The maximum weight measured in the adults was 4685.0g (434.8± g), the minimum 2515.0g (434.8± g) with a mean weight of 3667.5g (Tab.2). The maximum weight of the subadults was 3649.0g (769.6± g), the minimum 1209.5g (769.6± g) with a mean weight of 2625.5g (Tab.3). The maximum weight of female hares was 4685.0g (751.6± g), the minimum 1209.5g (751.6± g) with a mean weight of 3530.5g. The maximum weight of male hares was 4019.5g (654.8± g), the minimum 1723.0g (654.8± g) with a mean weight of 3049.3g. 48.6% (n=17) of the adults and 47.0% (n=8) of the subadults had a weight below the mean weight.

Necropsy findings

In 19 hares, visible lesions, i.e. multifocal raised white nodules, caused by intestinal coccidiosis could be found (Fig.2). Macroscopically 41.0% (n=7) of the adults and 62.5% (n=5) of the subadults with a weight beneath the mean weight, were severely affected by intestinal coccidiosis (seen as multifocal raised white nodules) or had mucous feces.

14 individuals, twelve adults and two subadults, showed unformed or even mucous feces, which can be a sign of enteritis. 50.0% (n=7) of these 14 hares had a weight below the mean weight of the respective group.

Four hares were infected with cysts of the tapeworm *Taenia pisiformis* (measuring approximately 1-1.5 cm in diameter) located on the intestine, liver, lungs and mesentery (Fig.3).

15.4% (n=8) of the hares had at least moderately swollen mesentery lymph nodes. Further changes were found in individual hares: fibrinous epicarditis; pitted and irregular surface of both kidneys; adhesions between the liver and the diaphragm;
perihepatitis and cysts in the liver and one approximately 1x3x1cm large abscess in the right cervical area.

Pathohistological findings
Eight organs were routinely examined: brain, intestine, mesentery lymph nodes, kidney, spleen, heart, lung and liver. Few organ samples from the 52 examined hares were missing: one intestinal sample, one heart sample, four samples of brain, one liver sample, four lymphnode samples and six samples of spleen. The absence is explained by damage due to the shooting and subsequently no samples could be collected in these cases.

96.0% (n=50) of the hares showed pathohistological abnormalities, in at least one organ. Solely 4.0% (n=2) were without any visible pathohistological abnormalities in the examined organs. 54.0% (n=28) of the hares showed pathohistological changes in more than one of examined organs. 4.0% (n=2) of the hares showed pathohistological changes in five out of eight evaluated organs.

Gastrointestinal tract
Out of 51 examined intestinal slides, 88.2% (n=45) showed pathohistological abnormalities, which were classified to be at least moderate.

The most common pathohistological finding in all 52 hares was a chronic lymphoplasmacytic enteritis. It was seen in 56.9% (n=29) of the investigated hares. From the total number of 51 intestinal samples, 25.5% (n=13) showed no histological signs of a coccidian infestation. The coccidiosis and the grade of infestation were classified using the number of coccidia found and the inflammatory reaction seen in the intestinal sample.

75.0% (n=27) of the 36 adult hares were affected by coccidia, out of which 13.9% (n=5) were classified as severely affected by the parasite. Furthermore, 75.0% (12 out of 16) of the subadult hares were affected by coccidia and 25.0% (n=4) were classified as severely affected (Fig.4).

Of the nine hares severely affected by coccidia, 66.7% (n=6) showed a weight lower than the calculated mean weight of 3667.5 g (for adults), respectively 2625.5 g (for...
subadults). 100.0% (n=9) of the severely affected hares showed also signs of an at least moderate chronic enteritis (Fig.5+6). One out of these nine (11.1%) hares was additionally infested with intestinal nematodes (Fig.7). 80.4% (n=41) of 51 hares were rated with at least a mild chronic enteritis. 39.2% (n=20) of the hares were infested with intestinal worms. Out of those 20 hares, 60.0% (n=12) suffered from at least a moderate worm infestation.

**Lymph nodes**
25.0% (n=12) of the 48 hares showed lesions classified as moderate or severe. 27.1% (n=13) showed signs of a histiocytic cellular infiltration. Out of these 13 affected hares, four (30.8%) were evaluated as severely affected. Also the adjacent vessels were affected in two cases and special stains were made (Fig.8). Eleven out of 48 (22.9%) showed signs of a purulent lymphadenitis. 6.2% (n=3) showed an eosinophilic cell infiltration in the lymph nodes. Mesenteric lymph nodes of two animals (number 21 and 33), were stained using Ziehl-Neelsen based on the suspicion of acid-fast bacteria. The Ziehl-Neelsen stain was negative in both cases. Further Gram, Giemsa and modified Ziehl-Neelsen stains were performed. The modified Ziehl-Neelsen staining showed a positive result but the attempt to identify *Mycobacteria sp.* by PCR was negative (Fig. 9).

**Brain**
6.2% (n=3) out of the 48 examined animals showed pathologic changes. Two of the hares were affected with a moderate perivascular lymphocytic infiltration combined with perivascular edema and a moderate multifocal lymphoplasmacytic to granulomatous encephalitis. The third affected hare showed signs of a moderate perivascular edema together with moderate multifocal lymphocytic encephalitis. Immunohistochemistry (IHC) of the brain and the kidney was performed in three animals (number 4, 31, 32) based on the suspicion of an infection with *Encephalitozoon cuniculi*. All three were positive (Fig. 10+11).
Kidney
52 kidney samples were examined, out of these 13.5% (n=7) showed changes in histopathology. Five (9.6%) cases of multifocal moderate chronic interstitial nephritis were determined. In one case tubulus regeneration with sclerosis were seen and in another one a moderate interstitial lymphoplasmacytic nephritis.

Spleen
8.7% (n=4) out of 46 examined spleens were diagnosed with pathologic abnormalities. Three (6.5%) had signs of moderate to severe purulent splenitis, with moderate to severe infiltration of heterophils. The fourth showed a moderate follicular splenomegaly/hyperplasia.

Liver
43.1% (n=22) of the 51 examined hares showed significant (classified as at least moderate) abnormalities. 49.0% (n=25) of all examined hares showed a multifocal lymphocytic hepatitis (mild changes included), one of them was classified as severe. 29.4% (n=15) of the 51 examined hares had signs of a multifocal mixed-cellular hepatitis.

Two hares demonstrated a cyst adenoma of the biliary duct in the liver and one hare had a biliary duct cyst.

Different organs (spleen, liver and kidney) of hares number 8, 49, 26 were stained with Congo-Red confirming amyloidosis. Two (8, 26) out of three animals had a positive reaction in the organs tested (Fig.12).

Heart
In the course of the pathohistological examination, two animals out of 51 (4.0%) showed abnormalities in the heart. One animal showed signs of a mild to moderate multifocal lymphocytic myocarditis, the second had signs of a moderate perivascular edema.
Lungs
There were no significant abnormalities in the 52 examined lungs, besides bleedings due to shooting wounds.
5. Discussion

The decreasing European brown hare population on Pellworm is nothing unique per se since a general decline of the hare population has been recognized in Europe during the last decades (PETROV, 1976; CHAPMAN and FLUX, 1990; MITCHELL-JONES, 1999; SMITH et al., 2005). The fact that the island does not inhabit predator mammals (BORKENHAGEN, 2011) makes the task to find reasons for the decline more demanding, as one of the most important reasons of hare population declines, the fox, must be disregarded (PEGEL, 1986; HOFFMANN, 2003; BORKENHAGEN, 2011).

The importance of parasites in population declines has been discussed; on the one hand parasites among wild animals must be seen as something normal and on the other hand parasites can, direct or indirect, be a cause of death. Since the aim of the parasite is to reproduce, the survival of their host is crucial. Therefore hares can often be massively infested without showing any severe symptoms or impacts on survival. But parasite infestation can also lead to severe secondary infections and it can be hard to fully determine whether the reason of death is due to the secondary infection or due to the parasites (HACKLÄNDER et al., 2006).

Among the 52 examined hares from Pellworm we found a coccidian infestation rate of 75.0% (n=39). This corresponds with HACKLÄNDER et al (2006) who assumed the hare population to be infested between 62.0-99.0% and with other publications describing the average infestations rate to be 70.0% (KÖTSCHE and GOTTSCALK, 1990).

In a study concerning the parasite load in Schleswig-Holstein parasites were detected in 272 of 296 hares (92.0%) and the most frequently diagnosed parasite were coccidia. The different species found were: *E. semisculpta* (52.0%), *E.leporis* (29.0%), *E.robertsoni* (15.0%), *E.europaea* (6.0%), *E.hungarica* (0.3%), *E.stiedai* (0.3%) (BÖCKELER et al., 1994).

Also in a study from Polen, coccidia were the most frequently diagnosed parasites
with a prevalence of 48.6% (53 from 109) (SECK-LANZENDORF, 1997). In a publication from Sweden, the presence of coccidia was much lower than in the above mentioned publications, with a prevalence of 7.9% (n=5) among the examined hares from the mainland and 4.8% (n=2) among those from the Swedish island Ven (THULIN et al., 2012).

Samples of our hares were all collected in October. That could be one explanation for the high prevalence, as studies in wild rabbits in Thüringen have shown that, although a coccidian infestation can be found all year round, it is up to 1.7 times higher from June until October (NICEL, 1987). According to HACKLÄNDER et al. (2006) autumn is the period with the highest parasite prevalence in hares. It is explained through the often occurring high numbers of hares during autumn which leads to easier distribution of parasites. But it is not only the number of hares which makes it easier for the parasites to affect more hares. Harvesting in autumn leads to a reduction of habitat. This inevitably leads to hares living in closer proximity to each other. And a lack of food can also negatively influence the condition of the animals. Other seasonal conditions like: bad weather and the use of pesticides are factors that negatively influence the condition of hares (NICEL, 1987). CHROUST et al. (2012) think a lower percentage of parasite-free hares could be explained by the occurrence of habitats favorable for the intermediate hosts of the parasites. Wet weather conditions do also favor the occurrence of some parasites e.g. coccidia (ZÖRNER, 1996).

We did not see a difference in the number of infestations between adults and subadults. This is in contrast to studies from Austria were the total *Eimeria* spp. prevalence was 80.4%; adults 60.0% and subadults 97.9%. In the same study hares from Czech Republic were examined. Here the total prevalence was 79.6%, adults 64.6% and subadults 95.5% (CHROUST et al., 2012). The higher grade of affected subadults is often described due to an inferior immune defense of subadults (HACKLÄNDER et al., 2006).

However we found a difference in the manifestation grade of the coccidian infestations between subadults and adults. The percentage of subadults with a severe infestation was 25.0% (n=4) compared to 13.9% (n=5) in adults. These
results are not significant (P= 0.31). This finding could be explained by the above mentioned hypothesis of subadults having an inferior immune system. Differences between females and males are also discussed. The size of the parasite host matters, because a bigger host offers more space for parasites to live in and reproduce (HACKLÄNDER et al., 2006). The fact that female hares often are a bit bigger than the males could be a reason why they are affected by parasites more often (HACKLÄNDER et al., 2006). The female hares in our study were bigger, with a maximum weight of 4685.0g (751.6± g) and with a mean weight of 3530.5g. The male hares had a maximum weight of 4019.5g (654.8± g) and a mean weight of 3049.3g. Looking at the infestation grade of females and males, there was just a slight difference, but in fact females were affected a little more often by coccidia than the males, with 56.4% (n=22) to 43.6% (n=17) - but also not significant (P=0.75). However the total number of females without an infestation was higher compared to the total number of uninfested males, 61.5% (n=8) to 38.5% (n=5). Comparing the grade of infestation between female and male at different time periods have to be considered, as the energy available for defense against parasites varies. In females this is during reproduction and lactation due to the fact that leverets only depend on the mother’s milk for the first three weeks of life before they start eating solid food (HACKLÄNDER et al., 2002). In males it is the time before reproduction. Another factor able to influence the grade of infestation is the hormone status of the animal. HACKLÄNDER et al.(2006) described that estrogen in females has a positive impact, while testosterone in males has a negative impact on the immune system of the hares.

Our histological findings, namely a lot of micro- und macrogametes in the intestinal mucosa and in many cases extensive damaged epithelia cells, correspond with MCCULLOCH et al. (2004). They described their findings in hares affected with coccidia as:”…a mild catarrhal inflammation and goblet cell hyperplasia. Massive infection of the mucosa with the various developmental stages of Eimeria leporis, especially the macro-and microgametes sexual stages. There was also extensive destruction of the jejunal epithelial cells“.
*Eimeria leporis* is ranked as the most pathogenic coccidia (SCHNEIDER, 1978; BÖCKELER et al., 1994). Its pathogenicity has also been documented by MCCULLOCH et al. (2004) who found the first case of a haemorrhagic jejuno-jejunal invagination with necrotic intussusceptions caused by *E. leporis*. This coccidian species therefore is supposed to be a primary cause of death in hares. Other pathohistologic and pathologic studies done among rabbits which died due to enteritis revealed that intestinal coccidia infestation just harms individuals already affected by an intestinal disease, coccidia alone were unlikely to cause lethal diseases (MESHORER, 1976).

We found 39.2% (n=20) of the hares infested with intestinal nematodes. Out of these 20 hares, 60.0% (n=12) suffered from at least a moderate worm infestation. Females and males were affected equally, each 50.0% (n=10). But again the number of uninfested females was higher with 62.5% (n=20), compared to 37.5% (n=12) in males.

At necropsy cysts of Cysticercus pisiformis were found in four hares, but no adult tapeworms were found. In a study from Austria one case of Cysticercus infestation was found (CHROUST et al., 2012) and according to KÖTSCHE and GOTTSCHALK (1990) just 0.7% of hares in the German-speaking part of the world die of a cysticercosis, nonetheless it can come to epidemic losses among young animals. KÖTSCHE and GOTTSCHALK (1990) described that about 2.0-3.0% of hares in the German-speaking part of the world are tapeworm carriers. The total tapeworm infestation in a study done by CHROUST et al. (2012) was 10.4% in the subadult hares in Austria and 6.1% in subadults from Czech Republic. There were no tapeworms found in adult hares neither in Austria nor in the Czech Republic. The total infestation grade was 4.4% in Austria and 2.9% in the Czech Republic (CHROUST et al., 2012). On Föhr, the neighbor island to Pellworm, were parasite-load was investigated, no tapeworms or cyst thereof could be found (pers. comm. KARTES). Thus tapeworms appear to be of minor importance for the health and survival of hares (CHROUST et al., 2012).
We found 17 (33.3%) hares infested with both coccidia and intestinal nematodes. BÖCKLER (1994) formulated a hypothesis about the mixed infestation load from helminthes and coccidia saying that parasites affect each other. Meaning that if a severe helminth infestation occurs, synchronously just a mild coccidian infestation can exist and vice versa. Among our 17 mix-infested hares this was the case in 47.0% (n=8), just one hare (5.9%) had both a severe coccidian infestation and a severe infestation with intestinal worms. The remaining 47.0% (n=8) hares had mild-mild or moderate-moderate infestations.

Coccidia are one of the most potent pathogen parasites among hares and in combination with intestinal nematodes it is seen as one of the major regulatory factors in hare populations (CHROUST, 1984).

To explore if parasites could have an influence on the decrease of a hare population BÖCKELER (1994) looked at the interaction between parasite-load and bodyweight. In that study there was no significant difference between the weight of individuals with or without parasites. And he concluded that it is improbable that the reason for the decrease were parasites. Another study showed the same results, with no influence of *Eimeria* spp. on the body weight of adult hares (CHROUST et al., 2012). Our results show that 48.1 % (n=25) of the hares had a weight lower than the calculated mean weight of 3667.5 g (adults) respectively 2625.5 g (subadults) and from these, 92.0% (n=23) were affected by parasites.

It is likely that parasites along with the occurrence of irregular strong winds in conjunction with wet periods are factors with a big influence on the wide variations seen in hare populations (WIEREN et al., 2006). The weather on Pellworm can be described as both wet and windy. HOFFMANN (2003) analyzed the weather using data from the Deutsche Wetterdienst (DWD) station on List/Sylt in the years from 1961-1990 and found the average yearly rainfall to be 747 mm and the average temperature 8.4 °C.

The most common pathohistologic finding in our 52 hares examined was a chronic lympho-plasmacytic enteritis, seen in 56.9% (n=29) of the cases.

In a rabbit breeding colony with chronic coccidiosis- and pasteurellosis-infected
rabbits the mortality rate due to diarrhea among the subadults was 20.0% and in adults 5.0%. The same mortality rate was seen in a second group of rabbits who also died of diarrhea but had no parasites (MESHORER, 1976). In a study of rabbits that died of enteritis, and apparently healthy rabbits from the same stable, the rectal contents were cultured on blood agar and two bacterial colonies were generally found: Bacillus spp. and Escherichica coli. Streptococci, Staphylococci and Proteus spp. were also found. Among the rabbits who died of an enteritis mainly E.coli was found, and among the healthy rabbits mainly Bacillus spp. (NIKKELES et al., 1976).

The rest of the pathological changes found must be seen as individual cases of disease without influence on the population decrease. Due to these results, reasons for the decline have to be found somewhere else. Researchers have found a positive correlation between temperature in autumn and a negative correlation with rain in spring (SMITH et al., 2005). It is also known that rain has a negative effect on juveniles during the first days of life (ZÖRNER, 1996; REICHLIN et al., 2006). In fact rain has been shown to have a strong effect on the numbers of hares. A negative correlation between the numbers of hares, the total yearly rainfall, the number of months when rain exceeds 100 mm and rain during the reproduction months has been shown (WIEREN et al., 2006). Even though the hare can reproduce all over the year (ZÖRNER, 1996) the months of March to May still are the most important months for an increase in the hare population (PEGEL, 1986). It is known, that through the introduction of biogas plants and an intensification of corn-production, there has been an overall intensification of the agriculture on Pellworm in the last years. One of the most common reasons for a decline described is the intensification of the agriculture (SMITH et al., 2005). The most probably reason for the decrease is an interaction between changes in agriculture leading to changes in the habitat and food availability, along with parasite infestations. To find the definitive reasons of the decrease in the hare population of Pellworm further investigations are needed.
6. Summary

As in many European countries a decrease of the local European brown hare population has been noted on Pellworm. This decrease had its high in the years 2006-2008. To find reasons for the decline a thorough health screening was started. The focus of this paper is the histopathological examination which was performed as one part of the study.

In October 2011 during the normal hunting season 52 hares were shot and dissected. Tissue samples were collected and fixed in 7% neutrally buffered formalin. Histological slides were made, stained with H&E stain and evaluated under a light microscope. Besides the H&E-staining, further special staining methods were performed when deemed necessary: Congo-Red-staining, Ziehl-Neelsen, modified Ziehl-Neelsen, Gram and Giemsa. A Polymerase Chain Reaction (PCR) for the detection of mycobacteria was performed.

The most common finding was a parasitic infestation with subsequent enteritis. 56.9% (n=29) of the investigated hares showed a chronic lymphoplasmacytic enteritis.

74.5% (n=38) of the hares showed signs of a coccidian infestation. The second common parasites diagnosed were intestinal nematodes, found in 37.2% (n=19) of the hares. Three hares were positive for *Encephalitozoon cuniculi* in Immunohistochemistry (IHC) of the brain and kidney.

Parasites can have an influence on the health and survival of affected hares. The partly high parasitic burden together with the enteritis is most likely one of the causes leading to the decline. But to be able to grasp the underlying cause further investigations are needed.
7. Zusammenfassung

Titel: Pathologische Auswertung von Feldhasen einer Insel und Vergleich der erhobenen Daten mit Literaturdaten vom Festland.


Ergebnisse: Die am häufigsten gefundenen pathologischen Veränderungen waren Parasitosen mit begleitender Enteritis. 56.9% (n=29) der untersuchten Hasen hatten eine chronische lymphoplastomzytäre Enteritis. Die am häufigsten auftretenden Parasiten waren Kokzidien, mit einer Prävalenz von 74.5% (n=38), gastrointestinal Würmer zeigten sich in 37.2% (n=19) der Hasen und damit am zweithäufigsten. Encephalitozoon cuniculi war in drei Hasen mittels positiver IHC-Färbung von Nieren- und Gehirnproben nachweisbar. Neben den angeführten Parasiten, wurden auch mittelgradige Hepatitiden und Lymphadenitiden gefunden. Alle anderen erhobenen
histopathologischen Veränderungen (Myokarditiden, Splenitiden, Splenomegalien, Nephritiden) waren nur bei einzelnen Individuen nachzuweisen.

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Tab. 1: Overview over the most common *Eimeria* species in the hare (NICKEL, 1987).

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Tab. 2: This table shows the weight in gram and the gender of the 35 adult hares. M=male, F= female.

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**Tab. 3:** This table shows the weight in gram and the gender of the 17 subadult hares. M=male, F=female.

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<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>f</td>
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</tr>
</tbody>
</table>

Average value: 2625.5  
Standard deviation: 769.6  
Maximum: 3649.0  
Minimum: 1209.5
Danksagung

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