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EPIZOOTIOLOGIC AND ECOLOGIC INVESTIGATIONS OF EUROPEAN BROWN HARES (*LEPUS EUROPAEUS*) IN SELECTED POPULATIONS FROM SCHLESWIG-HOLSTEIN, GERMANY

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ABSTRACT: From 1997–99 European brown hare (*Lepus europaeus*) population densities were estimated by spotlight surveys within different areas in Schleswig-Holstein, Germany. These areas showed a wide variation in local hare population densities. In addition, red fox (*Vulpes vulpes*) densities were estimated in 1997 by surveys of fox dens and litters. Sera of 321 hares (shot between 1998–2000) from four study areas were examined for antibodies against European brown hare syndrome virus (EBHSV) by enzyme linked immunosorbent assay (ELISA), *Yersinia* spp. (n=299) and *Francisella tularensis* (n=299) by western blotting, *Brucella* spp. by Rose Bengal test, and *Toxoplasma gondii* by Sabin-Feldman test (n=318). Tissue samples comprising lung, liver, spleen, kidney, heart, and adrenal glands were collected for histopathology. Liver (n=201) and spleen (n=201) samples were processed for the detection of *T. gondii*-antigen in tissue sections and 321 liver and spleen samples were investigated for EBHSV-antigen by ELISA. Furthermore, 116 hares were examined macro- and microscopically for lungworms. Significant negative correlations between hare and fox densities were found in spring and autumn 1997. Antibodies against EBHSV were detected in 92 of 321 (29%), against *Yersinia* spp. in 163 of 299 (55%), and against *T. gondii* in 147 of 318 (46%) hares. We evaluated the potential influence of origin and hunting season on exposure rates of hares using logistic regression analysis. A strong association between hare densities and exposure rates was observed for various agents. One hundred and eight of 201 (57%) hares were positive for *T. gondii*-antigen. All sera were negative for antibodies against *Brucella* spp. and *F. tularensis* and all lung samples were negative for lungworms. In conclusion, variation in red fox densities may have an impact on the hare populations examined and the infectious diseases we studied seem to play a subordinate role in the dynamics of European brown hare populations from Schleswig-Holstein.

Key words: Brucellosis, epizootiology, European brown hare syndrome, Germany, *Lepus europaeus*, yersiniosis, toxoplasmosis, tularemia, *Vulpes vulpes*.

INTRODUCTION

A general decline of European brown hare (*Lepus europaeus*) populations has been observed in several European countries since the 1960s (Petrov, 1976; Stubbe et al., 1994). Various reasons for this decline have been suggested (Seck-Lanzendorf, 1997); for example, changes in farm practices seem to have had an impact on the distribution of hares (Tapper and Barnes, 1983, 1986; Ahrens, 1996). In several European countries, studies have shown that there are significant variations in leveret survival (Pepin, 1989) and that reproductive success may be low under modern agricultural conditions (Pegel, 1986; Hansen, 1992). In Germany, predation by red fox (*Vulpes vulpes*) was suggested to be higher than in earlier decades. Investigations of the ecology of the red fox have shown an increase in the reproductive rate of at least 200%, and an increased lifespan from 288 to 657 days in the absence of rabies (Goretzki et al., 1999). Various infectious diseases of hares also may have a negative impact on local European brown hare populations (Seck-Lanzendorf, 1997).

The causative agent of European brown hare syndrome (EBHS) is a small (30 to

35 nm) icosahedral, non-enveloped virus (EBHSV) classified as a calicivirus by Ohlinger and Thiel (1991). European brown hare syndrome is characterized by acute hepatitis and hemorrhages of various internal organs (Poli et al., 1991) and its occurrence has been reported from many European countries (Frölich et al., 2001).

Pseudotuberculosis is one of the most important causes of death in hares and is caused by pathogenic strains of the genus *Yersinia.* The term pseudotuberculosis originally referred only to infection with *Y. pseudotuberculosis.* However, recent investigations by Wuthe and Aleksic (1997) showed that *Y. enterocolitica* O:2a,2b,3b,c biovar 5 ("the hare strain") and O:5,27 biovar 2/3 or O:3 biovar 5 can mimic the clinical presentation of pseudotuberculosis in hares. Therefore, it seemed appropriate to include *Y. enterocolitica* infections in the clinical picture of the pseudotuberculosis syndrome. The signs of pseudotuberculosis are mild to severe enteritis, enlargement of the spleen and various lymph nodes, and granulomatous nodules in several organs. In 1990–91, *Y. pseudotuberculosis* was isolated from 12 of 90 hares (13%) found dead in Schleswig-Holstein, Germany (Wuthe et al., 1995). In contrast, *Y. enterocolitica* was isolated from only 4% of these hares.

Tularemia, caused by *Francisella tularensis,* occurs naturally in lagomorphs and rodents, with ticks and other arthropods the main vectors, but infections of many other mammals and birds have been described (Mörner and Sandstedt, 1983; Selbitz, 1988). In the European brown hare, clinical signs of brief, severe apathy are followed by fatal septicemia (Mörner and Addison, 2001). In Europe, tularemia is endemic in Sweden and Austria (Mörner et al., 1988; Höflechner-Pöltl et al., 2000) and has been detected in a few areas in Germany (Knothe et al., 1959; Weber, 1994).

Brucellosis in hares is caused by *Brucella suis* biovar II (Thorne, 2001). Wild boars (*Sus scrofa*) and hares are considered to be the natural reservoirs. *Brucella suis* biovar II is also capable of infecting domestic pigs (Dedek, 1983). The infection in hares is either latent or involves the development of granulomatous nodules or abscesses in the testes, liver, spleen, lung and other tissues (Bisping and Amtsberg, 1988). According to Weber (1994), hare brucellosis is endemic in a few geographic regions in Germany.

Toxoplasmosis, caused by *Toxoplasma gondii,* is frequently found in hare populations and leads to an acute fatal disease in most cases. Transmission usually occurs by ingestion of food or water contaminated with oocysts from cat feces (Dubey, 1986). In areas where toxoplasmosis is endemic, the predominant lesions in hares are interstitial pneumonia, multifocal areas of hepatocellular necrosis, encephalitis, and moderate necrosis of lymphoid follicles of the lymph nodes (Christiansen and Siim, 1951).

Lungworm infection is one of the major parasitic diseases especially in young hares and is caused by *Protostrongylus pulmonalis* and *P. tauricus* (Kutzer, 1997). Both species cause pleuropneumonia and may lead to a general loss of condition especially if secondary bacterial infections occur.

Our objectives in this study were to determine whether there is an association between European brown hare population densities, and 1) exposure to selected infectious agents (EBHSV, *B. suis, Yersinia* spp., *F. tularensis, T. gondii*), 2) detection of EBHSV and *T. gondii* antigen, 3) occurrence of *Protostrongylus* spp., 4) histopathologic findings, and 5) fox densities. We chose Schleswig-Holstein state in Germany because wide variation in local hare population densities has been observed in this region.

MATERIAL AND METHODS

The four intensive study areas were located in the most northern German state, Schleswig-Holstein (Figs. 1, 2). Study areas I–III were located in different biogeographic units according to Meynen et al. (1962). The main factor

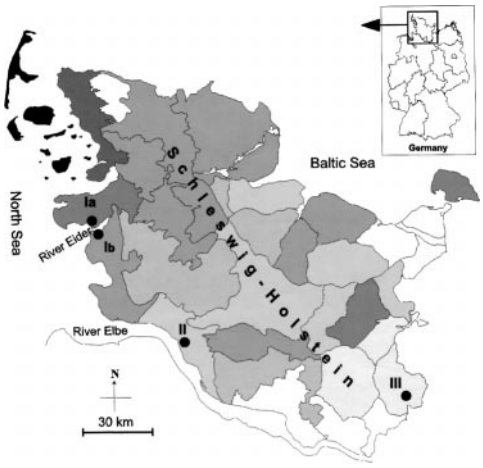

FIGURE 1. European brown hare (*Lepus europaeus*) densities in Schleswig-Holstein (Germany) in autumn 1997. □ 0–5/km², ▨ 5–10/km², ▧ 10–15/km², ▨ 15–▤ 20/km², ▦ 20–25/km², ▩ 25–30/km², ■ >30/km², □ no data available. Location of the four study areas in Schleswig-Holstein; Ia: Katinger Watt, Ib: Wesselburener Koog, II: Elbmarschen, III: Lauenburg.

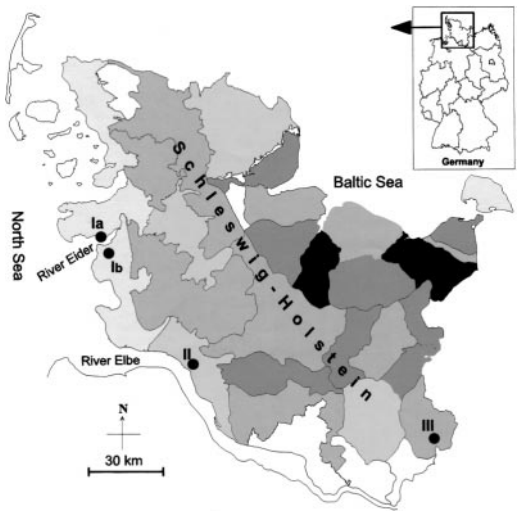

FIGURE 2. Estimated red fox (*Vulpes vulpes*) densities in Schleswig-Holstein (Germany) in spring 1997. □ 0.5–1/km², ▨ 1–2/km², ▧ 2–3/km², ▨ 3–4/km², ■ >4/km², □ no data available. Location of the four study areas in Schleswig-Holstein; Ia: Katinger Watt, Ib: Wesselburener Koog, II: Elbmarschen, III: Lauenburg.

for selection of these study areas was considerable differences in hare population densities. Study areas Ia (Katinger Watt; 54°17′N, 8°52′E) and Ib (Wesselburener Koog; 54°14′N, 8°53′E) represented North Sea marshes. These lowland marshes with clay soils are characterized by large fields divided by open drains. Study area Ia is separated from Ib by the river Eider which is about 1 km wide. Study area Ia north of the river is characterized by crops, sown grasslands, and patches of woodland. Study area Ib is located on the southside of the river Eider in an area of intensive vegetable cultivation. Study area II (Elbmarschen; 53°49′N, 9°29′E) is part of the river Elbe marsh area. The land use is similar to area Ia. Study area III (Lauenburg; 53°35′N, 10°49′E) is part of a hilly region in the east of Schleswig-Holstein, characterized by large fields of rape and crops divided by hedges. Thirty percent of this area is covered by woodlands.

From 1997–99 European brown hare abundance was estimated by spotlight survey within different biogeographic units according to Pfister and Rimathe (1979) modified by Pegel (1986). This was performed in February/March (spring counting) in 40 non-overlapping areas and in October/November (fall counting) in 37 non-overlapping areas in Schleswig-Holstein. The four intensive study areas were located within these areas (Fig. 1).

In 1997, fox population densities were estimated by surveys of fox dens and fox litters according to Lloyd (1980) (Fig. 2). The total number of fox litters was determined by local hunters and the average fox group size was calculated to be 6.4 (2.5 adult foxes and 3.9 young foxes).

During the hunting seasons (15 October–15 January of the following year) in 1998, 1999, and 2000 a total of 321 hares were shot in study areas I–III. Blood was collected from all hares, centrifuged, and sera were stored at −20 C. Tissue samples including lung (n=321), liver (n=321), spleen (n=321), kidney (n=321), heart (n=133), and adrenal glands (n=115) were collected. All samples were sent (at 4 C) within 1–3 days to the Institute for Zoo and Wildlife Research (Berlin, Germany).

Pathogenicity of *Y. pseudotuberculosis* and *Y. enterocolitica* has been linked to *Yersinia* outer proteins (YOPs). Two hundred and ninety-nine serum samples were tested for specific antibodies against YOPs by *recom*Blot *Yersinia* IgM/IgA (Mikrogen, Martinsried, Germany) according to the manufacturer's description with minor modifications. The test is based on five recombinant proteins (YOP M, H, D, E, and the V-antigen, a proposed regulator). The V-antigen is encoded on the 64 kDa *Yersinia* virulence plasmid and is present in pathogenic strains of *Y. pseudotuberculosis* and *Y. enterocolitica,* respectively. Briefly, recombinant proteins were

submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE) and blotted on nitrocellulose. Western blots were incubated with diluted serum samples, anti-rabbit-immunoglobulin G (IgG)-horseradish-peroxidase conjugate (DAKO Diagnostik GmbH, Hamburg, Germany) and ready-to-use 3,3′-diaminobenzidine tetrahydrochloride (DAB) substrate. Anti-YOP antibody-YOP complex was visualized as a brownish band on the blot stripes.

Three hundred and twenty-one serum samples were examined for antibodies against *Brucella* spp. by the Rose Bengal test (Davies, 1971). The test was performed as a plate-agglutination-test with Bengalrose dyed *B. abortus*-antigen. *Brucella abortus* shares epitopes with *B. suis* (Bisping and Amtsberg, 1988). *Brucella*-antigen, positive and negative control sera were obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine (Berlin).

Two hundred and ninety-nine serum samples were examined for antibodies against *F. tularensis* by western-blotting. An extraction of the lipopolysaccharid (LPS) fraction from the live vaccine strain of *F. tularensis* (ATCC 29648) was used as antigen (Grunow et al., 2000). Briefly, PAGE was performed using a 4–20% separating tris-glycine gel (Novex, Frankfurt, Germany) and electrophoretically separated proteins were transferred onto nitrocellulose membranes. The strips were incubated with a polyvalent goat anti-rabbit IgG horseradish peroxidase conjugate (DAKO, Glostrup, Denmark) and developed with 3,3′,5,5′-tetramethylbenzidine (Seramun, Dolgenbrodt, Germany). Samples were considered positive if they showed the typical LPS rope ladder.

Three hundred and twenty one liver and spleen samples were tested for EBHSV antigen, whereas the corresponding serum was tested for specific antibodies against EBHSV. We used EBHSV-antigen and antibody-blocking enzyme-linked immunosorbent assay (ELISA) test kits according to the methods of Frölich et al. (2001). Briefly, the EBHSV-antigen ELISA microplates (Maxisorp, Nunc-Roskilde, Denmark) were coated with rabbit anti-EBHSV IgG, and the supernatants from the spleen material were analyzed as quadruplicates. Following overnight incubation and washing, the wells were reacted with immunosorbent treated guinea pig anti-EBHSV hyperimmune serum in two wells and with normal guinea pig serum in the residual two wells, and finally all wells were incubated with rabbit anti-guinea pig peroxidase conjugate (Denmark code P141, DAKO Diagnostik GmbH) and orthophenylene-diamine substrate (Sigma-Chemie GmbH, Deisenhofen, Germany). The results were read at 490 nm on an MTF 10 spectrophotometer (Wissenschaftlicher Gerätebau, Berlin). Test samples that deviated significantly >3 standard deviations (SD) from the mean of the negative controls in a dilution of ≥1:10 were considered positive. A modification of the antigen ELISA was used for the detection of blocking antibodies against EBHSV in a competitive assay. Briefly, serum samples were added to anti-EBHSV IgG precoated wells, followed by a predetermined dilution of a known EBHSV-antigen suspension. After a new incubation period the previously described procedure for the antigen ELISA was followed. Sera that deviated <3 SD from the mean of negative control sera in a dilution of ≥1:10 were considered antibody positive.

Three hundred and eighteen serum samples were examined for antibodies against *T. gondii* by the Sabin-Feldman test (Sabin and Feldman, 1948) at the Robert-Koch-Institute (Berlin). Live *Toxoplasma*-tachyzoites stain blue with alkaline methylene blue dye. Antibodies against *T. gondii* are detrimental to live tachyzoites and the cells appear unstained. Briefly, live tachyzoites were mixed with different dilutions of hare sera. Additionally, active human serum was added and the mixture was incubated for 1 hr, stained and examined by light-microscopy. Samples were considered antibody positive if 50% of the *Toxoplasma*-tachyzoites remained unstained in a dilution of ≥1:16 (Janitschke, 1979).

In 1999, 116 hare carcasses were examined macro- and microscopically for lungworms.

Tissue samples comprising lung (n=321), liver (n=321), spleen (n=321), kidney (n=321), heart (n=133), and adrenal glands (n=115) were collected for histopathology. The specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 2–4 μm, stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) (Hotchkiss, 1948; McManus, 1948). Tissue samples of spleen and liver from 191 animals were processed for the detection of *T. gondii* antigen. Deparaffinized tissue sections were coated with polyclonal anti-*T. gondii* rabbit serum (Quartett Immunodiagnostica, Berlin) and reacted with biotinylated porcine anti-rabbit serum and an avidin-alkaline phosphatase complex using naphthol/fast red as chromagen (Biotechnologie GmbH, Berlin). Sections were counterstained with Harry's hematoxylin (Sigma-Aldrich, Taufkirchen, Germany) and examined microscopically. Sections from identical tissues incubated with rabbit control serum served as negative controls.

We evaluated the potential influence of origin (four study areas) and hunting season

TABLE 1. Minimum and maximum hare densities in four study areas of Schleswig-Holstein (Germany), 1997–99.

Study area	Hares per km^2	
	Spring	Fall
Ia	25.8–31.7	28.2–43.0
Ib	58.5–81.2	120.0–126.7
II	7.8–10.2	6.0–9.4
III	2.5–7.2	3.8–5.2

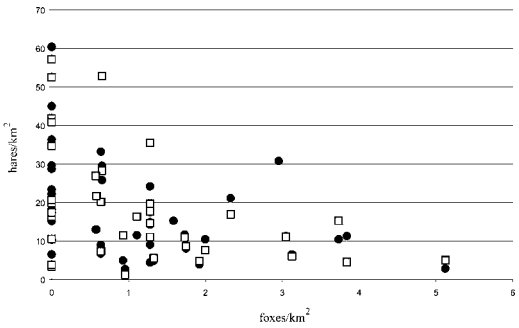

FIGURE 3. Association between European brown hare (*Lepus europaeus*) and red fox (*Vulpes vulpes*) densities in the different study areas in spring and autumn 1997. ● = hare/fox densities in spring, □ = hare/fox densities in autumn.

(1998–2000) on exposure rates of hares using logistic regression analysis (Hosmer and Lemshow, 1989). Adjusted standardized residuals in contingency tables were calculated to identify the categories responsible for significant associations between nominal variables (Everitt, 1977). Pfanzagl's test (Bortz et al., 1990) was used to verify a potential monotonous trend in a 2 × r contingency table. Kendall's coefficient of concordance W (Bortz et al., 1990) was used to check for the parallel course of exposure rates across study areas over 3 yr. The degree of simultaneous presence or absence of two specific antibodies in the same animal was measured by the phi-coefficient (Bortz et al., 1990). Spearman's correlation coefficient was used to analyze potential relationships between hare and fox densities. The significance level was set to $\alpha=0.05$. SPSS 9.0 (SPSS Inc., Chicago, Illinois, USA) and Sample Power (SPSS Inc.) were used for all statistical calculations.

RESULTS

Population densities of European brown hares varied within the different study areas (Table 1). For each year, population densities of hares declined in the sequence of the study areas Ib-Ia-II-III. The association between hare densities and fox densities in the different study areas is shown in Figure 3. Significant negative correlations between hare and fox densities occurred in spring (Spearman's correlation coefficient rho=−0.51, $P=0.001$, $n=39$) and in autumn 1997 (rho=−0.48, $P=0.003$, $n=37$).

Antibodies against YOPs were detected in 163 (55%) of 299 serum samples. Annual numbers and percentages of occurrence in the study areas are shown in Table 2. The logistic regression analysis ($n=299$) showed that prevalence depended on both study site ($P<0.001$) and year ($P<0.001$). For a more detailed analysis, the significant variables were separately tested using standardized residuals. The spatial differences were due to a high number of positive samples in study area Ib (standardized residual SR=7.2), and a low number in area II (SR=−6.6). In terms of temporal variation, less than expected positive animals occurred during 1999 (SR=−4.9) and a significantly higher than expected number in 2000 (SR=6.1). However, the temporal profiles of exposure rate in the four study areas were not synchronized (Kendall's W=0.188, $P=0.472$; Table 2). In order to test for a potential association between exposure rates and hare densities across study areas, we ordered the four exposure rates by decreasing hare density (Ib, Ia, II, III). Pfanzagl's test confirmed a monotonous trend in the resulting 2×4 table ($P<0.001$).

TABLE 2. Frequency of hares seropositive to *Yersinia* outer proteins during three hunting seasons. Number of seropositive reactors/number of samples tested.

Study area	1998	1999	2000	Total positive
Ia	11/37	10/20	26/30	54%
Ib	33/34	14/20	20/20	91%
II	2/28	4/45	23/30	28%
III	10/11	6/14	4/10	57%
Total	56/110	34/99	71/90	55%

TABLE 3. Frequency of hares seropositive to European brown hare syndrome virus during three hunting seasons. Number of seropositive reactors/number of samples tested.

Study area	1998	1999	2000	Total positive
Ia	14/40	2/20	23/30	43%
Ib	13/40	0/20	18/20	39%
II	6/40	2/45	11/30	17%
III	0/12	1/14	2/10	8%
Total	33/132	5/99	54/90	29%

Hence, hare density was positively associated with the exposure rate.

The distribution of EBHSV-antibody-positive hares is shown in Table 3. Antibodies against EBHSV were detected in 92 (29%) of 321 sera. Logistic regression (n=321) revealed significant differences in exposure rates between study areas (likelihood ratio test, $P<0.001$) and years ($P<0.001$). More positive samples than expected were found in areas Ia (SR=3.6) and Ib (SR=2.3) and less than expected in areas II (SR=−3.6) and III (SR=−2.3). Differences between years were due to more than expected positive samples in 2000 (SR=7.8) and a below-average number in 1999 (SR=−6.2). Temporal profiles of exposure rate were synchronized across study areas (Kendall's W=0.81, P=0.039). Thus, differences between study areas were caused by different levels of exposure of synchronous profiles. We found a strong positive association between hare density and exposure rate of the regions (Pfanzagl's test, $P<0.001$). EBHSV-antigen was only detected in one hare from study area Ia in 2000.

In total, antibodies against *T. gondii* were found in 147 (46%) of 318 serum samples (Table 4). The exposure rate increased from 1998 to 2000 in all study areas. In study area III, no antibody-positive hares were detected in 1998. However, in 2000 90% were seropositive. In the logistic regression analysis (n=318) exposure rates differed significantly between years ($P<0.001$), but not between study areas (P=0.674). Detailed analysis showed many antibody-positive animals in 2000 (SR=9.3) and a very low number in 1998 (SR=−8.2).

Toxoplasma gondii-antigen-positive hares are shown in Table 4. Antigen was detected in a total of 108 (57%) of 191 samples. In 70 (37%) of 191 hares *T. gondii*-antigen and antibodies against *T. gondii* were found. Furthermore, 38 (20%) of 191 hares reacted positive for *T. gondii*-antigen but negative for antibodies against *T. gondii*. Using logistic regression analysis (n=191), we found significant variation in antigen prevalences across study areas (P=0.001), but not between years (P=0.069). Differences between the study areas were due to more than expected positive samples in region Ib (SR=3.4).

A significant association was found between EBHSV-antibodies and *T. gondii*-antibodies, EBHSV-antibodies and *Yersinia*-antibodies and between *T. gondii*-antibodies and *T. gondii*-antigen (Table 5).

Nineteen hares (6%) had macroscopic

TABLE 4. Comparison of the frequency of hares with antibodies against *Toxoplasma gondii* (Ab) to those in which antigen was detected by immunohistochemistry (Ag) during three hunting seasons. Number of positive animals for each test/number of samples tested.

Study area	1998		1999		2000		Total positive	
	Ab	Ag	Ab	Ag	Ab	Ag	Ab	Ag
Ia	12/40	7/19	8/20	4/12	26/30	13/20	51%	47%
Ib	1/39	14/20	12/20	13/16	18/20	11/13	39%	78%
II	11/39	5/13	17/45	19/34	26/30	8/20	47%	48%
III	0/11	2/6	7/14	4/9	9/10	8/9	46%	58%
Total	24/129	28/58	44/99	40/71	79/90	40/62	46%	57%

TABLE 5. Associations between exposure rates to various agents (European brown hare syndrome virus [EBHSV], *Toxoplasma gondii*, and *Yersinia* spp.). Phi coefficients (upper right values) for each pair of infections and the assigned significance values and sample sizes (lower left values).

	EBHSV antibodies	*T. gondii* antibodies	*T. gondii* antigen	*Yersinia* antibodies
EBHSV antibodies		0.160	0.045	0.245
T. gondii antibodies	0.004 (318)		0.249	0.099
T. gondii antigen	0.532 (191)	0.001 (191)		0.144
Yersinia antibodies	<0.001 (299)	0.088 (296)	0.053 (181)	

lesions in various organs. Eighteen (16%) of 115 hares from study area II and one (0.6%) of 170 from study area I.

Tissues from 321 hares were examined histologically. In 302 (94%) individuals the organs had no lesions suggestive of disease. In these samples the occurrence of moderate infiltrates of lymphocytes, plasma cells, and histiocytes in the portal triads of the liver (n=62, 19%), the interstitium of lungs (n=49, 15%), or the interstitium of kidneys (n=38, 12%) were interpreted as insignificant. Microscopic hepatic changes were present in 15 hares (14 hares from study area II and one hare from study area I) and consisted of foci of necrosis surrounded by eosinophils and few lymphocytes. In addition, nonsuppurative interstitial nephritis (n=2, 0.6%), bronchopneumonia (n=1, 0.3%), and miliary necroses in the spleen (n=1, 0.3%) were present in four hares from study area II.

All sera were negative for antibodies against *Brucella* spp. (n=321) and *F. tularensis* (n=299) and all lung samples (n=116) were negative for lungworms.

DISCUSSION

In central Sweden, Small et al. (1993) found an inverse relationship between red fox and hare densities over a period of 29 yr. Using predator exclusion experiments on two different Swedish islands (Ranön and Bergön), Marcström et al. (1989) concluded that predation by red foxes was the limiting factor for mountain hare populations (*Lepus timidus*). Higher hare densities were found following a decrease of foxes after rabies infections in Germany (Spittler, 1972) or sarcoptic mange in Sweden (Dannel and Hörnfeldt, 1987). In Schleswig-Holstein, red fox populations have generally increased (Anonymous, 1998) probably due to various reasons such as rabies vaccination, reduced hunting pressure, and an increase in food resources. We found a significant negative correlation between hare and fox densities in our investigation areas. Therefore, variations in fox densities may have an impact on European brown hare populations in Schleswig-Holstein.

Antibodies against *Yersinia* spp. were found in 163 (55%) of 299 sera. This is in contrast to findings from other parts of Germany where seroprevalences in hares ranged from 13–17% (Schellner, 1977; Wuthe et al., 1995; Eskens et al., 1999). These differences might be explained by failure to diagnose *Y. enterocolitica* infected animals in previous investigations due to laboratory techniques based on LPS as antigen and a possible increase of *Yersinia* infections in European brown hares. Most antibody-positive sera were found in study area Ib with the highest density of hares. *Yersinia* spp. are transmitted directly or indirectly via contaminated food or water (Knapp and Weber, 1982) and the results of our statistical analyses demonstrate that exposure rate is strongly associated with hare density. Infection with *Yersinia* may promote other diseases and vice versa. This is supported by a weak but significant association between EBHSV- and *Yersinia*-antibodies. Our data showed that path-

ogenic *Yersinia* spp. are widely distributed as they occurred in all study areas.

Tularemia is endemic in several European countries including Germany (Selbitz, 1988; Weber, 1994). In 1959, Knothe et al. (1959) reported permanent natural foci in northern and central Germany. However, recently, antibodies against *F. tularensis* were not detected in hares from central Germany (Eskens et al., 1999) and all sera tested in our study were negative.

Hare brucellosis occurs only sporadically in Germany but is endemic in some European countries (Hubalek et al., 1993; Quaranta et al., 1995; Pilaszek et al., 1996). *Brucella suis* biovar II is maintained within hare populations but occurrence of *Brucella* in free-ranging hares reflects their previous or current contact with infected wild boars or infected domestic pigs. Dedek (1983) reported hare brucellosis in regions where brucellosis in wild boars was enzootic. A possible explanation for our negative findings may be that domestic pigs are mainly kept indoors and wild boars are rare in Schleswig-Holstein.

Antibodies against EBHSV were present in 29% of hares. Most antibody-positive reactors were detected in 2000 in all study areas; however, EBHSV-antigen was detected in only one hare. This virus is highly contagious and mainly transmitted by respiratory or orofecal routes. Most antibody-positive hares were found in study area Ia in a high density population; the statistical analysis revealed that hare density was strongly positively associated with exposure rate to EBHSV.

A high prevalence (66%) of *T. gondii* infection was found. Forty-six percent of hares had antibodies against *T. gondii* and 20% had tachyzoites but were seronegative. Hares positive for *T. gondii*-antigen but seronegative may not have had adequate time for developing antibodies. All hares with liver necrosis were negative for *T. gondii* by immunohistochemistry. Exposure of hares depends on exposure to feces from infected feral domestic cats or free-ranging felids sharing the same habitats. In our study areas, human population density is high, and therefore roaming cats are probably also present in high numbers (exact data about domestic cat densities are not available). In Germany, the seroprevalence of *T. gondii* in domestic cats varies between 45–74% (Ribbeck, 1992). In south and central Sweden, antibodies against *T. gondii* were not detected in any of 176 sera from European brown hares (Gustafsson and Uggla, 1994) and Poli et al. (1987) reported low prevalence (6.4%) in Tuscany (Italy). Seroprevalences of 23% and 45% were found in Romania (Elias, 1966) and in the former Czechoslovakia (Havlik and Hübner, 1958), respectively. Therefore, our results (46%) are consistent with the findings from former Czechoslovakia. In Swedish studies, occurrence of acute toxoplasmosis in free-living European brown hares has been reported to be between 10 and 12% (Borg, 1961; Gustafsson et al., 1988). In our study, most hares appeared to develop antibodies and clinical disease was not found.

Lungworms were not detected in any hare which is in concordance with a previous report by Kwapil (1993). Lungworms depend on snails as intermediate hosts which require specific habitat conditions (Haupt and Stubbe, 1990); our study areas may lack of these habitats. In other areas in Germany, lungworm prevalence varied between 2–44% (Gräfner et al., 1967; Gottschalk, 1973; Gottwald, 1973).

Most hares had no microscopic lesions in internal organs. This is probably because these hares were shot and presumably clinically normal. Nineteen hares (6%) had macroscopic lesions in several organs and microscopic hepatic changes were present in 5% without evidence of a particular disease. Although foci of hepatic necrosis were surrounded by eosinophils and lymphocytes we could not demonstrate parasites in these lesions. Kwapil (1993) reported liver lesions of unknown etiology in 20% of hares examined. Sixteen percent of the hares from study area II had severe lesions in various organs and

this may indicate the occurrence of an unknown infection in this area.

In conclusion, variation in red fox densities may have an impact on the hare populations in Schleswig-Holstein. Our study presents a comprehensive health assessment of shot hares from several populations in three successive years. European brown hares are exposed to a number of infectious agents (EBHSV, *Yersinia* spp., *T. gondii*). Undetected significant losses of hares in these areas can be excluded due to the present intensive monitoring between 1998–2000. Taken together, the infectious diseases we studied seem to play a subordinate role in regulation or limitation of European brown hare populations within Schleswig-Holstein.

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