Institut für veterinärmedizinische Untersuchungen Mödling[1], AGES, 2340 Mödling, Österreich; Pathologisches Labor[2], Forschungsinstitut für Wildtierkunde und Ökologie, Veterinärmedizinische Universität Wien, 1210 Wien, Österreich

Retrospective analysis of necropsy findings on the European brown hare *(Lepus europeus)* in AGES Mödling between 2018 and 2023, with a focus on tularaemia

L. Palasser[1a], R. Posch[1], S. Revilla-Fernández[1b], A. Kübber-Heiss[2c] and Z. Bagó[1d]*

ORCID: A) 0009-0005-6881-2819
B) 0009-0004-7309-3495
C) 0000-0001-6096-3835
D) 0000-0001-5897-5263

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Summary

The European brown hare (EBH) can carry various diseases, including zoonoses such as brucellosis, tularaemia, pasteurellosis, pseudotuberculosis and staphylococcosis. The hare is small game and a food source and it shares its habitat with humans and farm animals, so it is important to survey the population for diseases. We report a retrospective investigation of the pathomorphological and microbiological findings of 218 EBH carcasses that were sent to AGES Mödling between 2018 and 2023. A large proportion of the specimens were submitted from the federal state of Salzburg (116/218). The data were categorized and sorted, then visualized and interpreted, with a focus on individuals that had tested positive for tularaemia. During postmortem analysis the animals most frequently showed alterations in the lower digestive tract (44.1 %) and the respiratory system (23.9 %). Signs of trauma

Zusammenfassung

Retrospektive Analyse der Sektionsbefunde von Feldhasen an der AGES Mödling zwischen 2018 und 2023 unter besonderer Berücksichtigung der Tularämie

Einleitung
Feldhasen können Träger verschiedenster Krankheiten sein, darunter auch Zoonosen wie Brucellose, Tularämie, Pasteurellose, Pseudotuberkulose und Staphylokokkose. Als Niederwild, lebensmittellieferndes Tier und stetiger „Nachbar“ von Mensch und Nutztier liefert die Überwachung von Erregern des Feldhasen wertvolle Daten.

Material und Methode
In dieser Studie wurden die pathomorphologischen und weiterführenden mikrobiologischen Befunde von 218 Feldhasenkadavern, welche zwischen 2018 und 2023 an die AGES Mödling eingesandt wurden, retrospektiv aufgearbeitet. Ein Großteil der Tiere stammte aus dem Bundesland Salzburg (116/218). Die gesammelten Daten wurden kategorisiert und sortiert, um daraufhin graphisch visualisiert und ausgewertet zu werden. Ein spezielles Augenmerk wurde auf die Tiere gelegt, welche positiv auf Tularämie getestet wurden.

Ergebnisse
Die untersuchten Tiere zeigten überwiegend pathologische Veränderungen im unteren Verdauungstrakt (44,1 %) und im Atmungstrakt (23,9 %). Häufig wurden Anzeichen für Trauma (33,9 %) und Septikämie (14,7 %) festgestellt. Die Prävalenz der Tularämie positiven Tiere lag bei 33,9 % (74/218). Diese Feldhasen zeigten vermehrt Milzschwellung (56,8 %) und Nekroseherde in der Lunge (35,1 %), in den Lymphknoten (17,6 %), Hoden (14,9 %) sowie in der Leber (12,2 %). Nach erfolgreicher kultureller Anzucht konnten 65 dieser Tiere dem *Francisella tularensis* Biovar I (32) oder dem Biovar II (33) zugeordnet werden.

***E-Mail:** zoltan.bago@ages.at

(33.9 %) and septicaemia (14.7 %) were common. The prevalence of tularaemia was 33.9 % (74/218) but there was no consistent, significant increase in the number of cases from 2018 to 2023. Splenomegaly (56.8 %) and necrotic lesions in the lungs (35.1 %), lymph nodes (17.6 %), testicles (14.9 %) and liver (12.2 %) were frequent in hares positive for *Francisella tularensis*. Sixty-five isolates were subtyped as either biovar I or biovar II, with an unexpectedly high number of cases of biovar I (32/65). This biovar is usually endemic to western Austria but appears to have spread eastward. Further research is needed to evaluate the extent to which biovar I is established in eastern Austria.

Schlussfolgerung

Die Anzahl an Tularämie positiven Feldhasen war, aufgrund der nicht-repräsentativen Stichprobe, höher als die für Österreich erwartete Prävalenz. Dennoch konnte zwischen 2018 und 2023 kein konsistent signifikanter Fallanstieg festgestellt werden. Überraschenderweise waren etwa die Hälfte der Tularämie-Fälle dem Biovar I zuordenbar. Biovar I scheint sich geographisch weiter nach Ostösterreich auszubreiten. Weitere Studien sind notwendig, um zu evaluieren, inwiefern sich Biovar I bereits in den östlichen Bundesländern Österreichs etabliert hat.

Abbreviations: *B. = Brucella*; DIC = disseminated intravascular coagulation; EBH = European brown hare; EBHS = European brown hare syndrome; EBHSV = European brown hare syndrome virus; *F. = Francisella*; LISA.lims. = Laboratory Information and Management system; *M. = Mannheimia*; MRSA = Multi-resistant *Staphylococcus aureus; P. = Pasteurella*; RHD = Rabbit haemorrhagic disease; RHDV = Rabbit haemorrhagic disease virus

Introduction

The European brown hare (EBH) *Lepus europaeus* (Pallas 1778), is a member of the family *Leporidae* in the order *Lagomorpha*. The species is common throughout Europe and is ecologically and economically valued by predators and hunters. In the hunting season 2022/23, approximately 100,000 European brown hares were hunted in Austria, the majority in Lower and Upper Austria, followed by Burgenland, Styria and Salzburg (Statistik Austria 2023). Living in close proximity to humans and being a food source, the hare can pose a serious public health risk. Thanks to its position in this ecological and cultural niche, the species may offer insights into the interface of diseases with other species. The surveillance of zoonoses such as brucellosis, tularaemia, pasteurellosis and pseudotuberculosis is a vital step in realising the concept of One Health, with its holistic view of human, animal and environmental health. The necropsy of wildlife proves an effective and resource-saving tool in this endeavour. Gross morphological examination allows targeted diagnostic investigations, e.g. molecular biological analysis. The approach is especially important in researching diseases of wildlife due to limitations in funding and resources.

The following paragraphs briefly introduce some of the most prevalent and/or significant diseases of the EBH.

Tularaemia

In Europe, tularaemia is primarily caused by *Francisella tularensis* subspecies *holarctica (F. tularensis)* (Foley & Nieto 2010). Due to differences in antibiotic resistance, the zoonotic pathogen is typed into biovar I or II, with biovar II resistant to macrolides such as erythromycin (Tomaso et al. 2005). Both biovars are found in Austria, with biovar I largely confined to western Austria. Biovar I appears to have come to Austria from Germany and Switzerland (Schroll et al. 2018).

The life cycle of *F. tularensis* and its interaction with the environment is complex and features a terrestrial and an aquatic cycle. In the terrestrial life cycle, lagomorphs, rodents and ticks have important roles in maintaining the chain of infection. In addition to these species, a plethora of other mammals, birds, amphibia and invertebrates can carry and spread the pathogen (Foley & Nieto 2010; Gyuranecz 2012). The bacterium also survives in soil and water (Hennebique et al. 2019). *F. tularensis* persists in the environment and in its hosts, forming local foci in suitable climatic conditions (Pikula et al. 2003). These foci are likely to change under the influence of climate change and will have to be continuously reevaluated (Sjöstedt 2007; Deutz et al. 2009). Tularaemia occurs sporadically in endemic areas, first with epizootic courses (Hestvik et al. 2015) and followed after some time by human cases (European Food Safety Authority and European Centre for Disease Prevention and Control 2023; Hušková & Hušek 2023). Considering the numerous ways in which the pathogen sustains itself, the overall prevalence seems surprisingly low (Telford & Goethert 2011). A survey (Tobudic et al. 2014) of over 500 Austrian civilians and military personnel found a seroprevalence of only 0.7 %. Nevertheless, the European Union One Health 2022 Zoonoses Report (2023) noted a significant increase in human tularaemia cases reported in Austria from 2018 to 2022. The trends in Germany and Finland were similar. In addition, the disease may be severely underdiagnosed (Hestvik et al. 2015; Faber et al. 2018; Appelt et al. 2020).

The most frequent presentation of tularaemia in patients is the ulceroglandular and glandular form. Infected patients present with fever, local lesions and lymphadenitis. Infection occurs via wounds, through bites from infected animals and ticks, or by ingesting the pathogen, for example through consuming undercooked meat of infected animals. Inhalation of the bacterium can lead to severe cases of pneumonia and result in hospitalization (Anda et al. 2007; Appelt et al. 2020). Tularaemia cases related to handling wildlife highlight the importance of measures to avoid infection, as well as the need to raise awareness of the disease in the general population and the medical community (Lang & Kleines 2012). In Austria, the most common routes of infection are contact with infected animals and through tick bites (Schroll et al. 2018; Seiwald et al. 2020; Markowicz et al. 2021). *Ixodes ricinus* and *Dermacentor reticularis* carry the bacterium (Gehringer et al. 2013), with a prevalence of *F. tularensis* of 2.8 % in *Dermacentor* ticks (Hubálek et al. 1998). However, there is little known about the current prevalence in ticks in Austria.

European brown hares succumbing to tularaemia show disseminated, demarcated, necrotic gross lesions with marginal hyperaemia in organs such as the lungs, pericardium and kidneys (Gyuranecz et al. 2010). The spleen, liver and bone marrow are also commonly affected. In the case of acute septicaemia, the animals may present with little or no morphological alterations except an enlarged spleen (Hestvik et al. 2017). The organs that most frequently exhibit lesions seem to be linked to the genetic clade of the bacterium (Origgi & Pilo 2016). Microscopic, granulomatous inflammation with central necrosis can be detected in the affected parenchyma and serosa (Gyuranecz et al. 2010) and there may be foci in the lymph nodes and the adrenal gland, among other organs (Hestvik et al. 2017).

Brucellosis

Brucella suis (B. suis) infections occur in various mammalian species. Although largely eradicated in domestic pigs in Europe, *B. suis* biovar II remains active in its natural reservoirs: the wild boar and the EBH (Godfroid et al. 2005; Díaz Aparicio 2013; Godfroid et al. 2013). If farm biosecurity measures fail and there is close contact with wildlife, the pathogen may be reintroduced into livestock, particularly into pigs (Godfroid et al. 2005; Díaz Aparicio 2013; Jamil et al. 2022). Outbreaks of *B. suis* lead to significant economic losses in the agricultural sector as they reduce reproductive rates and increase the number of abortions or weak offspring (Díaz Aparicio 2013). In Austria, the most recent outbreaks in domestic pigs were in 2017 and 2024 (Österreichische Agentur für Gesundheit und Ernährungssicherheit 2024). Although the bacterium is known as a zoonotic agent, *B. suis* biovar II is not considered as pathogenic to humans as other *Brucella* species (Godfroid et al. 2005; Godfroid 2012). Nevertheless, infections do occur and there were seven reported human Brucellosis cases in France between 2004 and 2016 caused by biovar II (Mailles et al. 2017), all related to the hunting or skinning of infected wildlife.

Brucellosis can be transmitted directly and indirectly via various body fluids. High concentrations of the bacterium can be found in aborted foetuses, placentae and lochia (Godfroid 2012). The pathogen primarily enters the host through the conjunctiva and the mucosal lining of the oronasopharynx and the sexual organs (Godfroid 2012; Godfroid et al. 2013), then spreads via macrophages throughout the body, causing pyogranulomatous inflammation (Zachary 2022) and giving rise to macroscopic, purulent-necrotic lesions in lymphatic tissue, spleen, liver, lungs and the male and female reproductive system (Gyuranecz et al. 2011a; Zachary 2022).

Pasteurellosis

Pasteurella multocida is common, not only in the environment but also in the upper respiratory tract of healthy animals. The incidence of clinical pasteurellosis tends to be linked to stressors, for example a weakening of the host's immune system, and to weather conditions (Speck 2012; Yang et al. 2022). The disease can present with purulent inflammation of the upper and lower respiratory tract and with conjunctivitis, otitis, endocarditis, metritis and skin abscesses through wound infections (Speck 2012). Pasteurellosis has zoonotic potential, although there are few cases of human pasteurellosis through interaction with wildlife. Nevertheless, close contact to animals seems to increase the probability of infection (Wilson & Ho 2013).

Pseudotuberculosis

Yersinia pseudotuberculosis and to some extent *Yersinia enterocolitica* (Mair 1973) is a frequent cause of mortality of hares (Frölich et al. 2003). Death rates can be especially high during the winter months in periods of food shortage and harsh weather conditions. The bacterium occurs in soil and water and can infect a broad spectrum of hosts. Its main natural reservoirs are birds and rodents but most susceptible species can be asymptomatic carriers, causing further spread of the pathogen (Mair 1973; Najdenski & Speck 2012). Wherever habitats and food sources overlap, cross-species transmission may occur. *Yersinia* species are mainly transmitted faecal-orally, invading the intestinal wall and Peyer´s Patches before spreading to the adjacent mesenteric lymph nodes, the liver and the spleen, where they form pyogranulomas (Martínez-Chavarría & Vadyvaloo 2015; Fratini et al. 2017). Hares may show lesions in various organs and lymph nodes (Frölich et al. 2003), with the liver and spleen apparently most consistently affected (Fratini et al. 2017). Lagomorphs also commonly present with lesions in the *sacculus rotundus* and the vermiform caecal appendix (Espinosa et al. 2020). Yersiniosis is a foodborne zoonotic disease that may cause gastroenteritis and mesenteric lymphadenitis in human patients, although the condition is usually self-limiting (Martínez-Chavarría & Vadyvaloo 2015).

Staphylococcosis

Staphylococcus spp. are part of the normal skin and mucosal flora of many animals (Speck 2012; Monecke et al. 2016). When the tissue barrier is breached, the facultative pathogen may lead to suppurative inflammation with predominantly cutaneous abscesses. In severe cases, the systemic spread of the bacteria may lead to disseminated intravascular coagulation (DIC) (Speck 2012). The member of the *Staphylococcus* family that causes most concern to public health is *Staphylococcus aureus*. The growing issue of antimicrobial resistance has given rise to studies on the role of wildlife in the spread of methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA has been observed in hares and its prevalence is being investigated (Loncaric et al. 2013, 2014; Monecke et al. 2016; Moreno-Grúa et al. 2020).

European brown hare syndrome and Rabbit haemorrhagic disease

European brown hare syndrome (EBHS) was first described in Sweden about forty years ago. The lagovirus European brown hare syndrome virus (EBHSV), family *Caliciviridae*, is closely related to Rabbit haemorrhagic disease virus (RHDV) (Nowotny et al. 1997), which was considered to infect only rabbits. However, the RHDV2 strain has jumped species and causes EBHS-like symptoms in other members of the *Leporidae* family (Le Gall-Reculé et al. 2017; Velarde et al. 2017; Neimanis et al. 2018). The numerous similarities between EBHSV and RHDV have led to proposals for a new classification system for lagoviruses (Le Pendu et al. 2017). Both EBHSV and RHDV2 are highly tenacious and contagious. The mortality rate is strongly dependant on the virus strain and can be especially high in naïve populations (Duff & Gavier-Widén 2012). Hares aged 50 days and younger are not susceptible to EBHSV (Duff & Gavier-Widén 2012). This characteristic may not be applicable to RHDV2, as rabbits as young as ten days were found to succumb to the virus (Velarde et al. 2017). Transmission occurs directly and indirectly, usually through the faecal-oral route. The virus then spreads systemically to reach its target organ, the liver (Duff & Gavier-Widén 2012). Infected hares perish from liver and multisystem organ failure (Marcato et al. 1991). Typical necropsy findings include congested organs, diffuse haemorrhages in parenchymatous and mucosal tissue and an enlarged, brittle liver. In the rarer case of chronic disease progression, there may be necrotizing tubulointerstitial nephritis and chronic hepatitis (Duff & Gavier-Widén 2012). In the peracute form of EBHS and RHD, animals may present with subtle or non-specific findings in necropsy (Syrjälä et al. 2005). Histologically, the liver reveals distinct periportal to lobular hepatocyte necrosis with heterophils, fatty degeneration, haemorrhages and varying degrees of adjacent dystrophic calcification. Stages of liver cirrhosis may also be present (Marcato et al. 1991; Fuchs & Weissenböck 1992; Syrjälä et al. 2005).

Other infectious diseases

Other infectious diseases of the EBH include syphilis, myxomatosis, toxoplasmosis and other parasitic infestations. Hare syphilis or treponematosis is caused by the bacterium *Treponema paraluisleporidarum*. Affected animals present with ulcerative lesions of the genital tract and the facial skin areas. Seroprevalence appears to be generally high in Europe (Knauf et al. 2024), with a value of 43.9 % in Austria (Posautz et al. 2014). The lack of lesions in the hares indicates a certain level of resistance to the pathogen in the Austrian population.

The usually lethal disease myxomatosis, caused by the Myxoma virus, typically affects rabbits rather than hares. Recent years have seen an upsurge in the number of cases, with the introduction of a new virus variant (Águeda-Pinto et al. 2019; Friedrich-Loeffler-Institut 2024). Infected animals present with swellings and inflammation in the facial and genital area.

The zoonotic agent *Toxoplasma gondii* affects various hosts and poses a serious health concern for expectant mothers. The protozoan is also a frequent cause of acute death in hares (Frölich et al. 2003). Infected animals may initially demonstrate changes in behaviour. During necropsy, they may show haemorrhagic enteritis and an enlargement of the spleen and mesenteric lymph nodes. There may also be diffuse necrotic to haemorrhagic lesions in the liver (Christiansen & Siim 1951; Sedlák et al. 2000).

Hares also commonly carry parasites including *Eimeria* sp., *Trichuris leporis*, *Trichostrongylus retortaeformis* and *Protostrongylus pulmonalis*. *Eimeria* sp. and *Trichostrongylus retortaeformis* potentially cause severe diarrhoea. *Protostrongylus* infections may cause parasitic pneumonia and can be a precursor for bacterial superinfection. *Trichuris leporis* is believed to have insignificant pathogenicity to its host (Chroust et al. 2012).

As the EBH harbours various diseases that are relevant to humans, livestock and wildlife alike, it is highly beneficial to monitor the species. Data on necropsy findings, as collected by AGES Mödling, may extend our understanding of the health status of the Austrian EBH population and of its role in the spread of pathogens to other animal species and humans.

Materials and Methods

The data of 218 deceased hares from 2018 to 2023 were retrospectively investigated and categorized. The pathological findings, including pathogen identification and metadata, were collected and provided by AGES Mödling. The local databank "LISA.lims" (Laboratory Information and Management system)" was interrogated to select cases for this study. The carcasses were submitted by veterinarians, local authorities and hunters from eight of the nine Austrian federal states. As Carinthia has its own federal veterinary institute, no cases were included from this federal state. A considerable proportion (166/218) of specimens were sent from the federal state of Salzburg, so the hares studied are not representative of the general Austrian EBH population.

Only hares fulfilling the following criteria were included:

- The animal was sent to AGES Mödling between 01.01.2018 and 31.12.2023.
- The carcass included the lungs, liver and spleen.
- Mummified and heavily decomposed individuals were excluded.
- A gross morphological examination was conducted.
- Tissue samples were tested for *F. tularensis* and *Brucella* sp. via bacteriological culture, by molecular methods or both.

The major (moderate and high grade) pathomorphological findings were noted. A pathological agent identified via culture or PCR was included if consistent with the necropsy of the animal. The pathological findings were grouped into the following categories of organ system:

- Respiratory and cardiovascular system (heart, lungs, thoracic cavity)
- Alimentary system (digestive tract and its glands)
- Urinary and reproductive system
- Integumentary system
- Multiorgan (more than three organ systems involved, or signs of sepsis observed, e.g. high-grade splenomegaly, diffuse haemorrhage, septicaemia indicating lesions in two organ systems or lesions in several lymph nodes)

Other organ systems, such as the nervous system, were excluded as insufficient diagnostic data were available.

We assigned the findings to the following groups: inflammation, systemic circulatory disease, metabolic disease, degeneration/regeneration, foreign body/substance, displacement and neoplasia. The final four groups comprised only a small number of findings and were combined into a single group named "others" to simplify data presentation.

The study also incorporated additional diagnostic measures, including histology, which was performed in 28 cases, and parasitological diagnostics such as flotation and sedimentation, which were conducted on six hares. Parasite stages in lung tissue were observed either via tissue scraping and microscopic viewing or macroscopically if adult *Protostrongylus* sp. were present. Other parasites that were morphologically detected during examinations were also included in the analysis. Bacterial culture was performed 107 times and PCR 200 times. The *F. tularensis* subsp. *holarctica* biovar classification was performed by the disc diffusion test using erythromycin (Gurycová & Výrosteková 1998). PCR was exclusively used to detect *Brucella* sp. and *F. tularensis*.

A real-time PCR based on the Tul4 membrane protein was used to detect *F. tularensis* directly from the samples (Matero et al. 2011). The subspecies in positive samples was identified either from the samples or from bacterial cultures using a DNA intercalating dye and a melting curve analysis based on the M19 marker sequence, which differs between *F. tularensis* subsp. *tularensis* and subsp. *holarctica* (Byström et al. 2005). A commercial *Brucella* sp. qPCR assay based on the IS711 was used for the differential diagnosis (BactoReal, Ingenetix, Vienna, Austria).

DNA sequencing was performed in five cases when PCR proved insufficient. Please note that the years 2020 and 2021 saw a gradual shift in diagnostic methodology from bacterial culture towards molecular biological techniques due to resource-related factors.

We also recorded data such as sex (male, female, juvenile, undetermined), body condition and intravital trauma.

Following categorization of the data as described above, we generated diagrams, figures and a map showing the tularaemia-positive cases. We used a logistic regression model (generalized linear model) and a linear temporal trend to examine the prevalence of tularaemia cases over time.

Results

We analysed the data from 218 European brown hares (see Table 1). The vast majority (166) of the cases were submitted from the federal state of Salzburg. The sex ratio was approximately even, with 98 male and 97 female individuals; 23 hares with undetermined sex were also included. Due to the retrospective design of the study, it was not possible to access data on their sex. Most (70.6 %, or 154) of the animals were found in a state of cachexia or were severely underweight, with only 27 animals noted as of ideal body condition and two as obese. We saw moderate to severe signs of intravital trauma in 74 individuals (33.9 %) and seven hares had been shot. Thirty-two (14.7 %) presented with signs of septicaemia.

We observed lesions in all of the categories of organs defined above, i.e. in the respiratory and cardiovascular system (14), the alimentary system (32), the urinary and reproductive system (1) and the integumentary system (1), along with 115 cases of multiorgan lesions. Nearly 10 % (21) of the cases showed major lesions in two or three organ systems. The most frequently observed combination, with 17 cases, was the respiratory and cardiovascular system with the alimentary system. Thirty-four carcasses (15.6 %) showed no major patho-

Tab. 1: Number of hares included in the study per year, with their sex and federal state of origin / Anzahl der in die Studie inkludierten Feldhasen pro Jahr, mit deren Geschlecht und Einsender-Bundesland

year	m	f	u	Sbg	Stmk	Vbg	OÖ	NÖ	T	Bgld	W	Σ
2018	30	24	6	42	6	6	3	2	0	0	1	60
2019	6	5	1	3	1	4	2	0	2	0	0	12
2020	29	23	9	56	3	1	0	1	0	0	0	61
2021	14	20	1	29	2	0	3	1	0	0	0	35
2022	11	10	3	19	3	1	0	0	1	0	0	24
2023	8	15	3	17	1	2	1	3	1	1	0	26
Σ	98	97	23	166	16	14	9	7	4	1	1	218

m = male; f = female; u = undetermined sex; Sbg = Salzburg; Stmk = Styria/Steiermark; Vbg = Vorarlberg; OÖ = Upper Austria/Oberösterreich; NÖ = Lower Austria/Niederösterreich; T = Tyrol/Tirol; Bgld = Burgenland; W = Vienna/Wien

Tab. 2: Relevant (moderate to severe) pathological findings recorded during the necropsy of 218 European brown hares from 2018 to 2023 / Relevante (mittel- bis hochgradige) pathologische Befunde, welche während der Sektion von 218 Feldhasen in den Jahren 2018 bis 2023 festgestellt werden konnten

organ/organ system	inflammation				systemic circulatory disease		metabolic disease		others		Σ
lungs	pneumonia	48	mucopurulent	6							59
			purulent	10							
			necrotising	5							
			granulomatous	1							
			verminous	26							
	bronchitis			7							
	tracheitis			2							
	pleuritis			2							
heart	carditis	5	myocarditis	1	heart failure	3	aortic calcification	1			12
			epi- & pericarditis	3	cardiomyopathy	2					
			pericarditis	1	pericardial effusion	1					
upper digestive tract	parodontitis			1							5
	jaw abscess			1							
	gastritis/gastric ulcera			3							
lower digestive tract	enteritis	108	sero-mucous	80					intestinal perforation	1	109
			haemorrhagic	28							
liver	hepatitis/cholangitis	4	parasitic	3	chronic congestion/fibrosis	2	jaundice	6	cirrhosis	4	16
			bacterial	1							
urogenital tract	interstitial nephritis			1					dystocia with uterine necrosis and urinary retention	1	4
	metritis			1							
	pyometra			1							
integumentary system	phlegmon			2							2
systemic	septicaemia			32			amyloidosis	4	leiomyosarcoma	1	39
									neoplasia (undefined)	2	

logical findings other than traumatic injury or poor body condition. Table 2 presents a detailed depiction of all major necropsy findings, broken down by organ. In the carcasses, 88.6 % of organ alterations were of an inflammatory nature, while 4.5 % resulted from metabolic diseases and 3.3 % from systemic circulatory diseases. The category "others" (comprised of degeneration/regeneration, foreign body/ substance, displacement and neoplasia) contained 3.7 % of pathomorphological findings.

The most frequently altered organs were the lower digestive tract with 44.3 % and the lungs with 24.0 %. With one exception, a hare that succumbed to a perforation of the intestinal wall, both organs invariably presented with inflammatory lesions. Alterations of the liver (6.5 %) and the heart (4.9 %) were less frequent. Other organs with moderate to severe alterations were the upper digestive tract (2 %), the urogenital tract (1.6 %) and the integumentary system (0.8 %). Overall, 15.9 % of observations were of a systemic nature.

Infectious diseases and pathogens

As shown in Table 3, 43.1 % [(96 total – 2 coinfections)/218] of hares were diagnosed with bacterial infections, 44 % (96/218) with parasitic infestation and 1.4 % (3/218) with viral infections. Due to changes in the methodology over the years, we cannot exclude the possibility that other viral and bacterial infections were undetected or undiagnosed, as we only tested specifically for brucellosis and tularaemia.

Bacterial infections

The most frequently detected bacterium in the EBH population was *F. tularensis* with 74 (33.9 %) positive individuals, followed by *Mannheimia granulomatis (M. granulomatis)* with seven cases and *Y. pseudotuberculosis* with six. Other diseases caused by bacterial agents were pasteurellosis, listeriosis and clostridiosis. Two *F. tularensis*-positive hares were coinfected with *Y. pseudotuberculosis* and *M. granulomatis* (see Table 3).

We detected four cases (1.8 %) of brucellosis in animals sent from Salzburg in 2018 and 2020 and from Upper Austria in 2018 and 2023. *B. suis* was detected three times by both culture and PCR and once using PCR alone.

Tab. 3: Prevalence of bacterial, viral and parasitic (irrespective of grade) diseases of the European brown hares included in the study / Prävalenz von bakteriell-, viral- und parasitär bedingten (unabhängig des Grades) Erkrankungen der in dieser Studie inkludierten Feldhasen

		Σ	%
bacterial	tularaemia	74*	33.9
	mannheimiosis	7*	3.2
	pseudotuberculosis	6*	2.8
	brucellosis	4	1.8
	pasteurellosis	3	1.4
	listeriosis	1	0.5
	clostridiosis	1	0.5
viral	EBHS	3	1.4
parasitic	lungworm infestation	46	21.1
	intestinal parasites**	27	12.4
	ticks/fleas	14	6.4
	dicrocoeliasis/fasciolosis	6	2.8
	capillariasis	2	0.9
	hepatic coccidiosis	1	0.5

* number includes two coinfections (tularaemia and mannheimiosis, tularaemia and pseudotuberculosis); ** without further differentiation to species/ * Anzahl inkludiert zwei Koinfektionen (Tularämie und Mannheimiose, Tularämie und Pseudotuberkulose);** ohne weitere Speziesdifferenzierung

Tab. 4: Tularaemia case numbers and prevalence per year and federal state of the European brown hare population / Tularämie Fallzahlen und Häufigkeiten bei Feldhasen pro Jahr und Einsender-Bundesland

year	tularaemia (N)	hare (N)	%	Sbg	Vbg	OÖ	NÖ	Stmk	T
2018	15	60	25.0	12	3				
2019	5	12	41.7	1	3	1			
2020	26	61	42.6	24			1	1	
2021	16	35	45.7	14		1	1		
2022	8	24	33.3	7					1
2023	4	26	15.4	4					
Σ	74	218	33.9	62	6	2	2	1	1

Sbg = Salzburg; Vbg = Vorarlberg; OÖ = Upper Austria/Oberösterreich; NÖ = Lower Austria/Niederösterreich; Stmk = Styria/Steiermark; T = Tyrol/Tirol

Table 4 shows 74 tularaemia cases broken down by annual prevalence and including the hares' state of origin. The disease was diagnosed 25 times by bacterial culture, 40 times by PCR and eight times using both methods. One EBH in the study presented with typical morphological findings but no bacteria were detected, probably due to the poor preservation of the carcass. A logistic regression model showed a significant (p=0.04) rise in tularaemia cases in the population in 2020 and 2021 compared to the reference year 2018. However, the results were not significant (p=0.943) when we conducted a linear temporal trend.

Successful culture allowed 65 of the 74 cases to be assigned to subtype biovar I (32/65) or biovar II (33/65). Nine isolates could not be assigned to a biovar. Figure 1 shows the geographic distribution of the two biovars. Figure 2 presents the results of necropsy and histology on the distribution of tularaemia-indicative lesions in positive animals. About half the animals presented with moderate to severe splenomegaly (predominantly pulpous hyperplasia) (42). Typical necrotic lesions were most frequently detected in the lungs, lymph nodes, testicles and liver. A selection of these necrotizing to necrosuppurative lesions are presented in Figures 3 and 4. Hares with bacterial coinfections were excluded as the lesions could not definitively be assigned as caused by *F. tularensis*. Nearly 20 % (14) of the carcasses presented without relevant pathomorphological alterations but several individuals (48) presented with enteritis. Considering the vast number of causes of enteritis, we could not conclude that it had resulted from tularaemia.

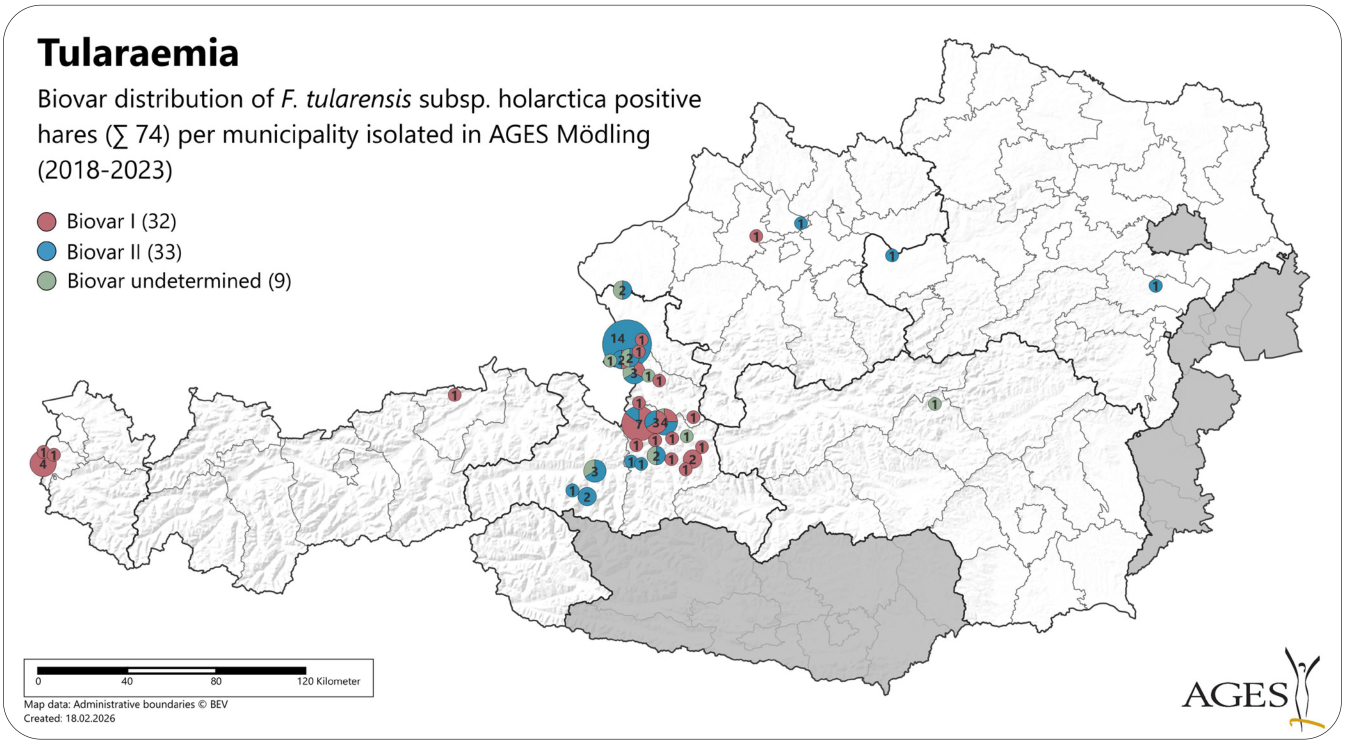

Fig. 1: Distribution of European brown hares tested positive for *F. tularensis* subsp. *holarctica* biovar I (32), biovar II (33) and undetermined cases (9) from 2018–2023. No cases were submitted from the federal states shaded in grey. / Verteilung der positiv auf *F. tularensis* subsp. *holarctica* Biovar I (32), oder Biovar II (33) getesteten Feldhasen, sowie nicht-klassifizierbare Fälle (9) von 2018–2023. Aus den grau schattierten Bundesländern stammten keine Fälle.

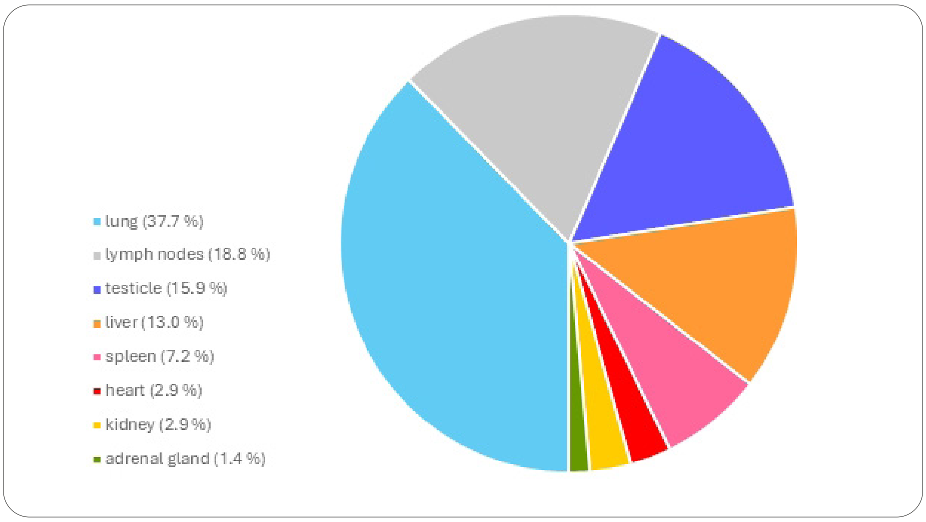

Fig. 2: Distribution of tularaemia-associated necrotic lesions in the 72 positive European brown hares (2 cases with coinfections were excluded) per organ/organ system / Verteilung der Tularämie assoziierten nekrotischen Läsionen der 72 positiven Feldhasen (2 Koinfektionen wurden nicht berücksichtigt) nach Organen/Organsystemen

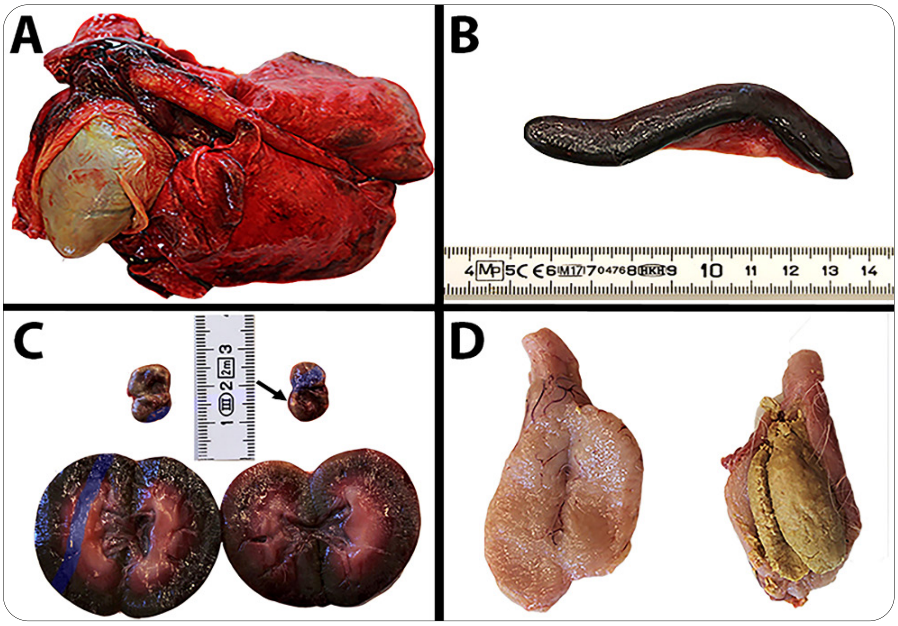

Fig. 3: Macroscopic findings in European brown hares that tested positive for *F. tularensis*: A) severe fibrinous epi/pericarditis (pericardial sack has been opened); B) severely enlarged spleen; C) multiple greyish necrotic lesions (arrow) in the adrenal glands; D) unilateral subtotal testicular necrosis / Makroskopische Befunde von auf *F. tularensis* positiv getesteten Feldhasen: A) hochgradige fibrinöse Epi-/Perikarditis (Perikard wurde eröffnet); B) hochgradige Milzschwellung; C) multiple gräuliche Nekroseherde (Pfeil) in den Nebennieren; D) unilaterale subtotale Parenchymnekrose in einem Hoden

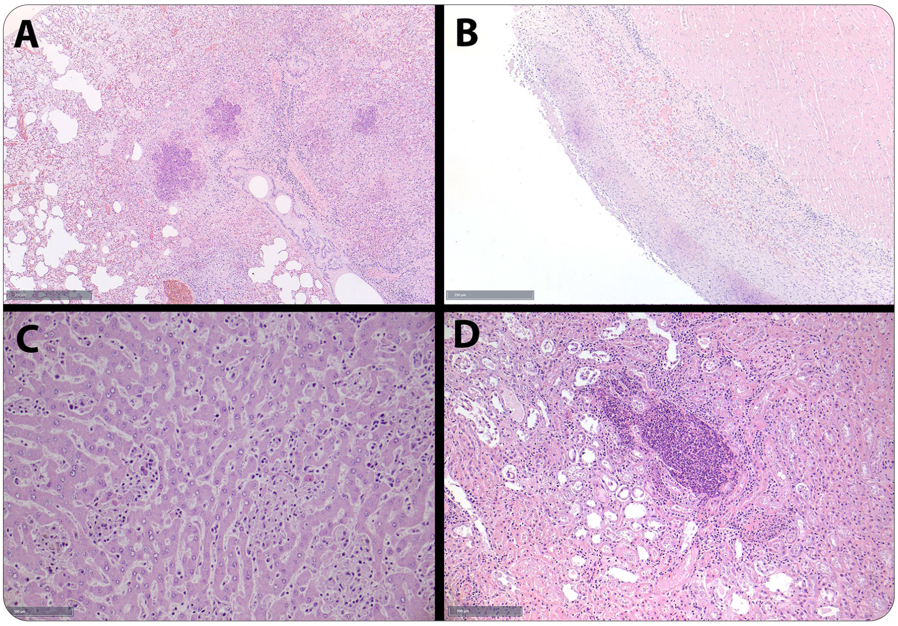

Fig. 4: Histological findings in European brown hares that tested positive for *F. tularensis*: A) oligofocal subacute necrosuppurative pneumonia, HE, bar: 250 μm; B) subacute to chronic necrosuppurative epicarditis, Haematoxylin-Eosin staining, bar: 250 μm; C) spotty necrosis of hepatocytes with slight lymphoplasmacytic inflammatory response, Haematoxylin-Eosin staining, bar: 100 μm; D) foci of subacute tubular mass necrosis with luminal accumulation of debris and neutrophils, Haematoxylin-Eosin staining, bar: 100 μm / Histologische Befunde von auf *F. tularensis* positiv getesteten Feldhasen: A) oligofokale subakute eitrig-nekrotisierende Pneumonie, Haematoxylin-Eosin staining, Balken: 250 μm; B) subakute bis chronische eitrig-nekrotisierende Epikarditis, Haematoxylin-Eosin Färbung, Balken: 250 μm; C) Gruppennekrosen von Hepatozyten mit minimaler, vorwiegend lymphoplasmozytärer Entzündungsreaktion, HE, Balken: 100 μm; D) fokale subakute tubuläre Massennekrosen mit intraluminaler Ansammlung von Detritus und neutrophilen Granulozyten, Haematoxylin-Eosin Färbung, Balken: 100 μm.

Viral diseases

In the period of the study, three (1.4 %) animals succumbed to EBHSV. Two individuals presented with macro- and/or microscopically indicative lesions. Samples from these two hares were sent for molecular biological analysis to the University of Veterinary Medicine, Vienna, where the diagnosis was confirmed. The third case was diagnosed morphologically.

Parasitic infestation

During necropsy, parasites were detected in 74 of 218 (33.9 %) cases. We observed adult, larval stages or eggs of *Protostrongylus pulmonalis* in nearly half (48 %) of the animals: 26 of them had pneumonia related to lung worms, 27 of them had intestinal parasites and 14 were infested with ticks or fleas. Nine animals had parasites in their liver: *Dicrocoelium dendriticum* (4), *Fasciola hepatica* (1), coinfection of *Dicrocoelium dendriticum* and *Fasciola hepatica* (1), *Capillaria* sp. (2) and *Eimeria* sp. (1). Due to inconsistencies in the precise identification of the parasites, the authors have excluded data on specific endoparasite agents.

Discussion

We explored the pathomorphological findings and additional diagnostic analysis of 218 EBH carcasses collected between 2018 and 2023. The data were retrospectively evaluated and presented visually. The results do not represent the general Austrian population of hares, as the cases sent to AGES Mödling for investigation were typically those with more obvious pathological alterations. Many deceased hares do not even reach official veterinary services as they are prone to being scavenged by predators such as foxes and birds of prey. In addition, passive surveillance heavily relies on the willingness of hunters to submit carcasses.

Nevertheless, passive surveillance databases such as these should not be disregarded. They can serve as an economical tool to provide a more thorough understanding of the health status of local wildlife populations. The surveillance of European brown hares is useful in early detection of disease outbreaks such as tularaemia (Hestvik et al. 2015), potentially allowing veterinary and public health services to react before diseases can spread to other species or to the general public.

The poor body condition of most of the animals investigated indicates a certain tendency for chronic disease progression rather than acute death. Additionally, more than 60 % presented with trauma related to predators or blunt force trauma, for example as experienced when hit by a vehicle. A similar study in Switzerland gave an even higher rate of 80 % (Haerer et al. 2001). Although such studies are based on a relatively small number of hares, large scale statistics support the percentages we observed. The Austrian hunting office collects annual data on wildlife found dead. In the years 2018–2023, an average of 28,000 deceased hares were recorded per year (Statistik Austria 2023). The statistics from 2014–2019 reveal that a consistently high percentage (mean 69.4 %) die from road traffic collisions (Statistik Austria 2020). The results are concerning, as the numbers of EBH in Europe are slowly dwindling (Smith et al. 2005).

As presented in Table 2, 68.3 % of the major pathological findings in gross examination were in the respiratory system and the lower digestive tract, with the most frequent alterations being pneumonia (19.5 %) and enteritis (43.9 %). A study in Schleswig-Holstein gave nearly identical results (Faehndrich et al. 2023b). Pathomorphological changes in other organ systems were underrepresented. Nearly 90 % (88.6 %) of the pathological alterations were assigned as inflammatory and the number of hares showing signs of septicaemia such as moderate to severe splenomegaly was correspondingly high (32/218).

We diagnosed 94 (43.1 %) cases of bacterial infections. The number is comparable to the results of other studies on the EBH in Austria (Deutz & Hinterdorfer 2000) but notably higher than the 12 % recorded in Switzerland (Haerer et al. 2001). More than two thirds (74) of the bacterial infections were caused by *F. tularensis*. The prevalence of tularaemia in the EBH ranges from 1.1 % (Runge et al. 2011) to 4.9–5.3 % (Gyuranecz et al. 2011b). In our biased population, we found a prevalence of 33.9 %. The results are analogous to those of a study on a tularaemia endemic region for several years, which concluded that an average of 30 % of hare deaths were caused by the disease (Stalb et al. 2017). It is possible that Austria, or at least the federal state Salzburg, where most carcasses in our study originated, has become an endemic focus for the disease. A counterargument is the relatively low seroprevalence in the Austrian EBH population. In an extensive study of 311 European brown hares in the north-eastern parts of Lower Austria, the seropositivity was only 7.07 % (Winkelmayer et al. 2005), even though Vienna and the city's vicinity are known local foci of tularaemia (Seiwald et al. 2020). Research between 1994 and 2005 (Deutz et al. 2009) predicted the possible geographical spread of tularaemia in Austria by 2035 based on climate change. Judging by our results, and in agreement with a previous suggestion (Seiwald et al. 2020), the predictions may have proven true more than ten years earlier than anticipated. The EFSA (European Food Safety Authority and European Centre for Disease Prevention and Control 2023) has reported significant increases in human tularaemia cases in Austria in recent years. Our data on the EBH do not reflect this trend. There was a temporary increase in cases in hares in 2020 and 2021 but no consistent long-term upsurge. The species is not considered the main source of human tularaemia infection in Austria.

As described in studies on tularaemia, *F. tularensis* subsp. *holarctica* biovar II represents the endemic biovar in Austria (Schroll et al. 2018). However, we found a surprisingly large number of biovar I cases. As expected, biovar I, except for one case from Upper Austria, was largely found in the western regions of Austria and biovar II largely in the eastern regions. As most of the European brown hares originated from Salzburg, the distribution of tularaemia in this state might be most accurately represented by our study. Figure 1 shows that the region seems to have become endemic for both biovars. Further studies will be needed to make any informed claims about the distribution of biovars in Austria's other federal states. Nevertheless, we are observing a tendency for biovar I to further spread east.

As summarised in Table 4, the animals most frequently presented with lesions indicative of tularaemia in their lungs, lymph nodes, testicles and the liver. Only 2.7 % showed foci in the heart or the kidneys. The latter findings are in contrast with the results of previous research (Gyuranecz et al. 2010), which concluded that the heart and the kidneys are major target organs of *F. tularensis*. In another study (Hestvik et al. 2017), 80 % of 49 hares that succumbed to the disease displayed an enlargement of the spleen, similar to the finding of splenomegaly in all 28 hares investigated (Origgi & Pilo 2016). As shown in Figure 2, we found an enlarged spleen in only about 60 % of cases. However, this percentage does not include cases with a minor swelling of the spleen. If we add such cases, the percentage rises to 97.3 %, representing the most consistent pathomorphological finding in *F. tularensis*-positive European brown hares.

The prevalence of *B. suis* in our population was 1.8 %, which was slightly lower than that found in other studies, e.g. 2.4 % (Haerer et al. 2001) and 5.6 % (Deutz

& Hinterdorfer 2000). A seropositivity of 3.54 % in the hare population of Lower Austria has been reported (Winkelmayer et al. (2005). Curiously surpassing the number of Brucellosis cases, seven cases of moderate to severe pneumonia related to *M. granulomatis* were observed. Little has been published on infections of the EBH with this pathogen. The bacterium has been suggested to be a commensal of the upper respiratory tract (Britton et al. 2017). We did not routinely sample the upper respiratory tract and cultured the bacteria exclusively from pathologically altered lungs.

Few European brown hares suffered from pseudotuberculosis (2.8 %) or pasteurellosis (1.4 %). Haerer et al. (2001) documented a prevalence nearly twice as high for both bacterial agents. A significant difference in the prevalence of pseudotuberculosis was recorded in the federal state of Styria with 23.5 % of EBH mortality associated with the bacterium (Deutz & Hinterdorfer 2000). However, due to the previously mentioned shift in methodology over the observed timespan, only a portion of the carcasses from this study were analysed by bacteriological culture. Therefore, hares infected, but lacking gross lesions during necropsy, may have been discounted as inconspicuous.

Approximately 1 % of the animals succumbed to EBHS virus, a figure similar to that reported previously (Deutz et al. 2000), despite the high seroprevalence within the Austrian hare population. As postulated for another EBH population (Faehndrich et al. 2023a; Faehndrich et al. 2023b), the numbers suggest a stable immunity to the disease.

The overall prevalence of parasite infestations (12.4 %) is rather low. Some data suggest that most European brown hares harbour intestinal parasites to some degree (Faehndrich et al. 2023a). We anticipated a low prevalence in our study as we primarily focussed on the macroscopic detection of gastrointestinal parasites instead of relying on more resource-intensive laboratory investigations such as flotation, sedimentation and the Baermann migration method. It is likely that we did not detect intestinal parasites in a large proportion of the hares. Table 2 shows that over a hundred individuals were diagnosed with moderate to severe enteritis. It is likely that a number of these cases can be attributed to intestinal parasites.

In contrast, we frequently detected parasites infesting parenchymatous organs with morphologically distinct lesions. Lung worm infestations were recorded in a fifth of the hares, in agreement with other authors (Deutz & Hinterdorfer 2000). We found trematodes in the liver of 2.8 % of the animals. The life cycle of trematodes is highly dependent on the availability of intermediate hosts, so it is important to consider the environmental conditions in the study area when interpreting the findings.

The retrospective study design, with its intrinsic flaws and the unrepresentative selection of EBH, resulted in the decision to refrain from statistical analysis of the results. There is a need for further, ideally prospective, studies with a representative population from each of Austria's federal states to draw definitive conclusions on the diseases of the EBH in Austria. Nevertheless, we can report compelling results on the distribution of tularaemia biovars in Austria. The data also highlight the fact that the EBH carries pathogens of zoonotic concern. The safe handling of carcasses and stringent biosafety measures are necessary. There is a case report (Lang & Kleines 2012) of two hunters who were infected with *F. tularensis* during the skinning of a hare; they experienced unnecessarily long delays from the onset of symptoms to diagnosis and treatment. Such incidents could be avoided by raising awareness of the diseases that may be carried by European brown hares, not only in the general public but also in the medical community. It recently (2022) became mandatory to report tularaemia-positive hares to official veterinary services in Austria and such cases are documented in the Verbrauchergesundheitsinformationssystem (VIS). Similar changes in legislation may support the gathering of epidemiological data on the disease in Austria and may also bring public attention to the potential risk of handling European brown hares without sufficient protective measures.

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Fazit für die Praxis:
Das Infektionspotential von Feldhasen sollte nicht unterschätzt werden. Der Europäische Feldhase stellt ein Bindeglied in der Infektionskette verschiedenster Krankheiten dar, darunter auch Zoonosen. Dies sollte man bei engem Tierkontakt stets bedenken. Um die Häufigkeiten dieser Krankheiten in Österreich besser zu erforschen, ist es unumgänglich auffällige Tiere zu erkennen und zur weiteren Untersuchung an einschlägige, veterinärmedizinische Institute wie z.B. die AGES Mödling einzusenden.

Conflicts of Interest: The authors declare no conflicts of interest.

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