Universität für Bodenkultur Wien
Department für Integrative Biologie und Biodiversitätsforschung
Institut für Wildbiologie und Jagdwirtschaft

Zoonotische Pathogene bei Tiroler JägerInnen und bei jagdbarem Wild

Dissertation zur Erlangung des Doktorgrades

Doctor rerum naturalium technicarum (Dr.nat.techn.)

an der Universität für Bodenkultur Wien

Eingereicht von

Monika Wetscher

Betreuer:

Univ. Prof. Dipl.-Biol. Dr. rer. nat. Klaus Hackländer
Universität für Bodenkultur Wien, Institut für Wildbiologie und Jagdwirtschaft

PD Dr. rer. nat. Georg Gerhard Duscher
Veterinärmedizinische Universität Wien, Institut für Parasitologie

Beratungsteam:

Univ.Prof. Dr.phil. Herbert Auer
Medizinische Universität Wien, Institut für Spezifische Prophylaxe und Tropenmedizin

PD Dr.med. Ninon Taylor
Paracelsus Medizinische Privatuniversität Salzburg, UK f. Dermatologie

OÄ Dr. med. Viktoria Faber
Paracelsus Medizinische Privatuniversität Salzburg, UK f. Innere Medizin III

Wien, März 2020

Alles, was gegen die Natur ist, hat auf Dauer keinen Bestand
Charles Robert Darwin 1809 - 1882

Inhaltsverzeichnis

I. Abstract II
II. Kurzfassung III
III. Danksagung IV
1 Einleitung und Hintergrund 5
2 Zielsetzungen und Fragestellung der Doktorarbeit 11
2.1 Aufbau der Rahmenschrift 11
3 Zoonotische Pathogene der zur Dissertation eingereichten Publikationen 1-3 13
3.1 1. Kapitel - Hunting poses only a low risk for alveolar echinococcosis 13
3.1.1 Kurzfassung 13
3.1.2 Wichtigste Ergebnisse der Untersuchung 13
3.1.3 Erkenntnis zur Fragestellung 14
3.1.4 Original Manuskript 14
3.2 2. Kapitel - Roe deer sera used for TBE surveillance in Austria 30
3.2.1 Kurzfassung 30
3.2.2 Wichtigste Ergebnisse der Untersuchung 30
3.2.3 Erkenntnis zur Fragestellung 30
3.2.4 Original Manuskript 31
3.3 3. Kapitel - Serological and molecular screening for tick-borne zoonotic pathogen *Babesia* spp. in hunters in Tyrol, Austria 45
3.3.1 Kurzfassung 45
3.3.2 Wichtigste Ergebnisse der Untersuchung 45
3.3.3 Erkenntnis zur Fragestellung 46
3.3.4 Original Manuskript 46
4 Zusammenfassung und Ausblick 59
5 Literaturverzeichnis 62
6 Liste der Publikationen und Präsentation der Autorin 71

I. Abstract

Tick-borne diseases show a wide variety of considerable human pathogenic potential, such as viral tick-borne encephalitis and human babesiosis caused by protozoa. Another parasitic worm disease dangerous for humans is infection with *Echinococcus multilocularis*. These pathogens therefore may affect public health. Austria is a comparatively small central european country with a high biodiversity and abundant wildlife. At the interface of wildlife habitats and human activities a range of pathogens can be transmitted from wild animals to domestic animals and to humans. Hunting activities and the resulting close contact with wildlife is repeatedly cited as a transmission risk. Wildlife is not only a reservoir for pathogens, but also serves as an indicator for the detection of risk areas.

In the context of this doctoral thesis, the occurrence and risk assessment of the above-mentioned pathogens will be discussed. For this purpose, both humans and wildlife were serologically and molecularly tested for the pathogens *Echinococcus multilocularis*, Tick-borne encephalitis virus and for *Babesia* spp.

The publications aim to substantiate and increase the current state of knowledge of zoonotic agents in Austria and to demonstrate their dangerous potential for public health by means of concrete questions. The available data could be used as valuable starting points for further research questions based on the existing empirical results

This work also demonstrates the need for further research in the veterinary and human medicine fields. Of particular importance would be the investigation of the pathogen *Echinococcus multilocularis* in pets in order to gain further insights into human infections.

Further investigation of further rarely studied pathogens such as theileria, anaplasma, babesia and rickettsia in human blood following a tick bite could too provide important information on epidemiological data on incidence and prevalence.

II. Kurzfassung

Durch Zecken übertragbare Krankheiten zeigen eine breite Vielfalt von beträchtlichem humanpathogenem Potential wie zum Beispiel die virale Frühsommer - Meningoenzephalitis und die durch Protozoen verursachte humane Babesiose. Eine weitere für den Menschen gefährliche parasitäre Wurmerkrankung stellt die Infektion mit *Echinococcus multilocularis* dar. Somit betreffen diese Erreger die öffentliche Gesundheit. Österreich ist ein kleines mitteleuropäisches Land mit einer hohen Artenvielfalt und reichlich Wildtieren. An der Schnittstelle von Wildtierhabitaten und menschlichen Aktivitäten kann eine Reihe von Pathogenen von Wildtieren auf Haustiere und auf Menschen übertragen werden. Jagdliche Aktivitäten und der dadurch nahe Kontakt zu Wildtieren werden immer wieder als Risiko dafür angeführt. Wildtiere sind nicht nur Reservoir für Pathogene, sie dienen auch als Indikatoren zur Detektierung von Risikogebieten.

Im Rahmen dieses kumulativen Dissertationsprojektes wird das Vorkommen und die Risikoeinschätzung der oben genannten Erreger diskutiert. Dazu werden sowohl Menschen als auch Wildtiere serologisch und molekularbiologisch auf den Erreger *Echinococcus multilocularis*, Frühsommer-Meningoenzephalitis Virus und auf *Babesia* spp. untersucht.

Die Publikationen zielen darauf ab den derzeitigen Wissensstand von Zoonoseerregern in Österreich zu untermauern bzw. zu erhöhen und deren gefährliches Potential für die öffentliche Gesundheit anhand konkreter Fragestellungen aufzuzeigen. Die vorliegenden Daten können als wertvolle Startpunkte für weitere Forschungsfragen auf Basis der bestehenden empirischen Ergebnisse herangezogen werden.

Diese Arbeit zeigt außerdem die Notwendigkeit weiterer Forschung im veterinärmedizinischen und im humanmedizinischen Bereich. Besondere Bedeutung käme der Untersuchung des Erregers *Echinococcus multilocularis* bei Haustieren zu, um weitere Erkenntnisse über die humanen Infektionen zu bekommen.

Weitere Untersuchungen von noch wenig erforschten Pathogenen wie z.B. Theilerien, Anaplasmen, Babesien und Rickettsien im Blut des Menschen nach einem Zeckenstich könnten wichtige Informationen über epidemiologische Daten bezüglich Inzidenz und Prävalenz liefern.

III. Danksagung

Schreiben musste ich meine Doktorarbeit zwar alleine, aber ohne die Hilfe vieler Menschen wären diese Erkenntnisse nicht zustande gekommen.

Mein herzlicher Dank gilt meinen Betreuern Prof. Dr. Klaus Hackländer, der sich meiner Doktorarbeit annahm und ich dadurch die Dissertation am Institut für Wildbiologie und Jagdwirtschaft absolvieren konnte und PD Dr. Georg Gerhard Duscher, der mir immer mit Rat und Tat zur Seite stand.

Weiters gilt mein Dank meinen Beratern Prof. Dr.Herbert Auer (Wien) und PD Dr. med. Ninon Taylor (Salzburg) für ihre hilfreiche Unterstützung.
Ein Dankeschön an meine Koautoren Prof. Dr. Julia Walochnik (Wien), OA Dr. Arno Michael Lechner (Salzburg) sowie an meine fleißigen Helfer Ina Kandil, Walburga Maderthaner, Dr. Sabine Rosenlechner, Dr. Michael Rosenlechner und Mag. Michaela Schachner für ihre Unterstützung und Hilfe.
Mein außerordentlicher Dank gilt OÄ Dr. Viktoria Faber, ohne deren Geduld und Verständnis in dieser anstrengenden Zeit ein solcher Arbeitsumfang niemals hätte gelingen können. Ihr Beistand als Beraterin und Mentorin, ihre kritischen Betrachtungen und zweckdienlichen Diskussionen gaben mir noch mehr Ansporn diese Arbeit zu schreiben und zu beenden. Vor allem aber ihr moralischer Beistand und menschlicher Halt haben mir Kraft und Mut zur Anfertigung und Vollendung meiner Dissertation gegeben.
Mein Dank gilt weiters meiner Familie und Freunden, die ich während dieser Zeit etwas vernachlässigte, da mir fast keine Freizeit blieb, aber deren Verständnis ich immer hatte.

Einleitung und Hintergrund

Zoonosen sind Infektionskrankheiten, die auf natürlichem Wege vom Wirbeltieren (Vertebraten) auf den Menschen übertragen werden (WHO 2018.) und eine erhebliche Bedrohung für die öffentliche Gesundheit darstellen (Taylor et al. 2001). Wo immer auch Menschen leben, ob in ruralen oder urbanen Gebieten, kann es zur Übertragung von Krankheitserregern von Tieren auf Menschen kommen (Morand et al. 2014).

Zoonoseerreger umfassen Bakterien, Viren, Parasiten und Pilze. Sie sind charakterisiert durch ein Tierreservoir, eine definierte Krankheit beim Menschen und durch bestimmte Übertragungswege (Murphy 1998). Zoonoseerreger können viele verschiedene Arten von Krankheiten bei Menschen und Tieren verursachen, die von leichter bis schwerer Krankheit bis hin zum Tod reichen. Manche Tiere können gesund erscheinen, selbst wenn sie Pathogene tragen, die Menschen krank machen können. Wissenschafter schätzen, dass mehr als 6 von 10 bekannten Infektionskrankheiten bei Menschen von Tieren verbreitet werden und 3 von 4 neuen oder aufkommenden Infektionskrankheiten bei Menschen von Tieren übertragen werden (CDC 2018, Spahr et al. 2017). Viele Freizeitaktivitäten erhöhen das Risiko einer Zoonose-Infektion (Pańczuk et al. 2019, Natarajan et al. 2017), nicht nur in ruralen sondern auch in urbanen und periurbanen Gebieten (Rizzoli et al. 2014). Österreich ist ein vergleichweises kleines mitteleuropäisches Land mit einer hohen Dichte an Wildtieren (Duscher et al. 2015). Durch die Jagdausübung ist ein Aufenthalt in freier Natur und ein naher Kontakt zu Wildtieren gegeben (Kuehn 2019, Petrovic et al. 2019, Nardoni et al. 2019, Robertson et al. 2019, Kmetiuk et al. 2019, Dobler et al. 2014, Gabrielli et al. 2014). An der Schnittstelle von Wildtierhabitaten und menschlichen Aktivitäten kann eine Reihe von Pathogenen von Wildtieren auf Haustiere und auf Menschen übertragen werden (Michalczyk et al. 2019, Duscher et al. 2015, Macpherson et al. 2005). Eine Früherkennung von Zoonosen durch Überwachung der Schnittstelle Mensch-Tier ist ein entscheidender Schritt zur Bekämpfung und zur Prävention von Zoonosen (Kotwa et al. 2019, Esser et al. 2019, Cull et al. 2019, Ahmed et al. 2019, Salyer et al. 2017).

1. Kapitel

Der Erreger *Echinococcus multilocularis* verursacht die humane alveoläre Echinokokkose (AE). *E. multilocularis* ist in Österreich endemisch (Auer 2006) und zirkuliert hauptsächlich in einem sylvatischen Lebenszyklus, wo der Rotfuchs (*Vulpes vulpes*) als Haupt- und Endwirt und Nagetiere wie z.B. *Microtus arvalis, Arvicola terrestris, Ondatra zibethica* als Zwischenwirte fungieren (Eckert et al. 2001, Oksanen et al. 2016). Zusätzlich besteht ein synantroper Lebenszyklus d.h. Rotfüchse adaptieren sich durch ihre hohe Anpassungsfähigkeit immer öfters in Städten (Gloor et al. 2001, Romig et al 2006, Robardet

et al. 2008). Dadurch findet eine Kontamination mit *E. multilocularis* in der urbanen Umwelt statt (Deplazes et al. 2011, Tylkowska et al. 2019). Ein ebenbürtiger Endwirt zum Rotfuchs ist der Marderhund (*Nyctereutes procyonoides*) und der Goldschakal (*Canis aureus*), Neozoen, die in Zukunft eine vermutlich zusätzliche Rolle bei der Verbreitung von *E. multilocularis* spielen (Duscher et al. 2017, Gherman et al. 2017, Oksanen et al. 2016). Haustiere, insbesondere Hunde, können ebenso als Endwirte fungieren. Die Kontamination erfolgt über die Ausscheidung infektiöser *E. multilocularis*-Eier (Eckert et al. 2004, Kern et al. 2004, Duscher et al. 2015, Karamon et al. 2019). Menschen erwerben die Infektion durch perorale Aufnahme der infektiösen *E. multilocularis*-Eier. Diese befinden sich im kontaminierten Boden, auf Nahrungsmittel oder auf Fellen (Eckert et al. 2004, Brunetti et al. 2010, Nagy et al. 2011). Der Mensch fungiert als akzidenteller Zwischenwirt. Das Metazestodenstadium von *E. multilocularis* zeigt ein tumorähnliches Wachstum - gleich einem malignen Tumor - und befindet sich überwiegend in der Leber (Kern et al. 2003, Tsaroucha et al. 2005, Ricken et al. 2017). Diese seltene, jedoch gefährliche Helminthozoonose führt unentdeckt und unbehandelt zum Tod (Piarroux et al. 2011, Chouhan et al. 2019). Durch die lange Inkubationszeit von bis zu 20 Jahren ist es ein sehr langsamer Verlauf und bleibt oft bis zum Auftreten von Symptomen unentdeckt (Brunetti et al. 2010).

Enzymimmunotest (ELISA), Westernblotverfahren (WB) und PCR stehen derzeit laborchemisch zur Abklärung von Krankheitsfällen und zur Überwachung des Therapieerfolges zur Verfügung. Diese Methoden werden ebenfalls für epidemiologische Untersuchungen zur Erhebung der Seroprävalenz sowie in der Präventivmedizin verwendet (Auer 2006). Für bildgebende Verfahren stehen die Positronen-Emissions-Tomographie und die Magnetresonanztomographie (PET/MRI) zur Prävention und zur Nachbeobachtung für das AE-Management zur Verfügung (Lötsch et al. 2017).

Eine Fall-Kontroll-Studie wurde 1997 in den westlichen Bundesländern Österreichs (Tirol und Vorarlberg) durchgeführt und zeigt die Jagd als unabhängigen Risikofaktor im Zusammenhang mit AE (Kreidl et al 1998). Auch weitere Studien postulieren, dass die Jagdausübung einen Risikofaktor eine AE zu erwerben darstellt (Eckert 1996, Deutz et al. 2003, Tiaoying et al. 2005, Schneider et al. 2013, Sadkowska-Todys et al. 2015).

In den letzten 35 Jahren traten in Österreich durchschnittlich 2 Krankheitsfälle pro Jahr auf (Auer et al. 2001), seit 2011 nehmen Fälle von AE in Österreich zu (Schneider et al. 2013). Im Jahr 2015 gab es 14 Fälle, von denen 7 in Tirol aufgetreten sind (persönliche Information, Auer, H. 2016).

2. Kapitel

Das FSME-Virus gehört zu den Flaviviren. Flaviviren sind von Arthropoden übertragene Viren. In Europa kommt nur der Erreger der Frühsommer-Meningoenzephalitis (FSME) vor. Bei den

Flaviviren hat sich in den meisten Fällen ein Zyklus zwischen Vertebratenwirt (Säuger, Vögel) und Überträger (Stechmücken, Stechfliegen und Zecken) herausgebildet, der für das Virus effizient und für die Reservoirwirte (Arthropodenvektoren) relativ unschädlich ist. Der tierische Vertebratenwirt zeigt häufig wenig Krankheitszeichen und übersteht die Infektion mit einer kurzdauernden Virämie gut. Während dieser Zeit infiziert sich der blutsaugende Überträger, der daraufhin das Virus mit dem Speichel ausscheidet und lebenslänglich infektiös bleibt. Nur Schildzecken (Ixodidae) sind in der Lage FSME-Viren zu replizieren und zu übertragen (Labuda et al. 1999; Agergaard et al. 2019). Der Mensch ist für das Virus eine Sackgasse und nimmt nicht an dessen Kreislauf teil (Kayser et al. 1993, Nah et al. 2019). Für den Menschen kommt als Infektionsweg – neben dem Zeckenstich – auch eine alimentäre Infektion z.B. durch rohe Ziegen-, Kuh- und Schafmilch in Frage (Holzmann et al. 2009, Brockmann et al. 2018). Ein seltener aber möglicher Übertragungsweg sind auch Aerosolinfektionen in FSME-Virus-Laboratorien (Horst 1991). FSME ist in allen neun Bundesländern in Österreich endemisch. Im Jahr 2014 beträgt die Durchimpfungsrate aller österreichischen Einwohner 85% (GfK Healthcare 2014). Es wird angenommen, dass deshalb zwischen 2000 und 2011 mehr als 4000 Menschen nicht infiziert wurden. (Heinz et al. 2013). Diese hohe Durchimpfungsrate ist für eine geringe Anzahl von humanen FSME-Fällen in Österreich mit einer Inzidenz unter 1 pro 100.000 Einwohner im Jahr 2009 bei einer Impfrate von 88% verantwortlich (Donoso Mantke et al. 2011; Heinz et al. 2013; Walder et al. 2008). Durch die hohen Durchimpfungsraten kommt es zu einem verzerrten Eindruck des FSME-Infektionsrisikos, es treten zwar weniger Fälle von FSME-Erkrankungen auf, doch die Gefahr einer Infektion bleibt für ungeimpfte Personen bestehen (Stefanoff 2013).

Die FSME-Verbreitungskarte für Österreich wird jährlich anhand der neu diagnostizierten humanen FSME-Erkrankungen aktualisiert (Baxter Healthcare GmbH, Wien). Die Zirkulation des FSME-Virus ist lückenhaft und stark abhängig von den unterschiedlichen Anforderungen an Vektor und Reservoirwirten, die wiederum von Habitatparametern wie Klima und Vegetation beeinflusst werden (Estrada-Peña et al. 2014; Randolph 2009). Daher wird die Identifizierung von Risikogebieten erschwert, da es problematisch ist, all diese kleinen Herde zu entdecken. Die Frühsommer-Meningoenzephalitis ist in Europa in den letzten Jahrzehnten ein wachsendes Problem für die öffentliche Gesundheit geworden (Süss 2011, ECDC 2018), der Verlauf der Erkrankung reicht von der moderaten bis zur tödlichen Meningoencephalitis (Kunze 2016). Das Ergebnis dieser Karte könnte aufgrund räumlich inhomogener Immunisierungsraten und des Fehlens von Daten aus den unverdächtigen moderaten Infektionen von menschlichen Patienten verzerrt sein. Um diese Probleme zu überwinden wurden zusätzliche Strategien entwickelt: nüchterne Zecken wurden in verschiedenen Ländern gesammelt und auf das Vorkommen von FSME-Viren untersucht (Dobler et al. 2011, Gäumann et al. 2010, Holbach und Oehme 2002, Kupča et al. 2010, Rieille et al. 2014, Süss

et al. 2004). Die Untersuchung der Prävalenz des FSME-Virus in nüchternen Zecken liefert wichtige Informationen in verschiedenen Regionen und zu verschiedenen Zeitpunkten. Allerdings sind diese Tests für eine detaillierte Risikoeinschätzung wenig geeignet, weil eine lückenhafte Verteilung der FSME-Herde auf sehr kleinen Flächen stattindet (Stefanoff et al. 2013). Engmaschige Kontrollen, welche zeitaufwändig und kostenintensiv sind, wären hier notwendig (Süss et al. 2004). In einigen Fällen kann die Viruslast in nüchternen Zecken sehr niedrig sein, sodass die Nachweisgrenze erst nach der - während des Saugaktes stattgefundenen - Replikation erreicht wird (Belova et al. 2012).

Bei gepoolten Proben kann eine geringe Viruslast unerkannt bleiben, sodass die tatsächliche Virusverteilung verfälscht wird. Auch der Vergleich von humanen FSME Erkrankungen und Prävalenznachweisen in nüchternen Zecken brachte für eine Kartierung von Risikogebieten keine aussagekräftigen Ergebnisse (Stefanoff et al. 2013). Zum Erhalt räumlicher Verteilungen wurden Indikatorarten wie Wild- oder Nutztiere identifiziert und versuchsweise eingesetzt (Stefanoff et al. 2013). Nagetiere dienen als natürliches Reservoir. Sie zeigen eine Serokonversion von etwa 2,6% (Achazi et al. 2011; Radda et al. 1971; Tonteri et al. 2011). Leider ist der Fang, die Probenentnahme und ein engmaschiges Untersuchen der Flächen sehr zeitintensiv, sodass in dieser Studie Nagetiere nicht untersucht wurden. Aus mehreren Gründen wurde das Rehwild als Indikatorart gewählt. Das Rehwild (*Capreolus capreolus*) kommt - außer in reinen Fels- und Gletscherregionen - ubiquitär in ganz Österreich vor (Reimoser et al. 2009). Rehwild ist sehr standorttreu und verbleibt in etwa innerhalb von 0,16 – 0,81 km^2 (Jeppesen 1990; Nosek et al. 1967; Radda et al. 1968b). Rehwild zeigt einen starken Befall von *Ixodes ricinus* (Kiffner et al. 2010; 2011; Vor et al. 2010). Symptomatische FSME-Infektionen wurden allerdings beim Rehwild bisher jedoch nie beschrieben (Nosek et al. 1967; Radda et al. 1968a, b). Auch für die weitere Übertragung spielt Rehwild keine Rolle, da die auftretende Virämie nach einem Zeckenstich im Reh zu gering ist. Rehe bilden aber Antikörper und können daher als Indikatorspezies durch FSME Antikörpernachweis im Serum verwendet werden Mehrere Studien zeigen, dass Rehwild eine sehr gute Indikatorspezies ist, um FSME Antikörper nachzuweisen (Gerth et al. 1995; Kiffner et al. 2012; Nosek et al. 1967; Radda et al. 1968a, b; Skarphedinsson et al. 2005).

3. Kapitel

Von Vektoren übertragene Krankheiten sind eine zunehmende Gefahr und Bedrohung für Menschen, Haus- und Wildtiere (Schmid et al. 2013, Duscher et al. 2015).

Der Aufenthalt in freier Natur führt zwangsweise zum verstärkten Kontakt mit Zecken und ihren Infektionserregern. JägerInnen zählen daher zu einer Risikogruppe (Dobler et al. 2014, Gabrielli et al. 2014). *I. ricinus* ist die in Österreich am häufigsten und am weitesten verbreitete Zeckenart (Stanek 2009) und wird bis in einer Höhe von 2500 m Seehöhe nachgewiesen (Sixl

et al. 1972). Wie im 2. Kapitel beschrieben, ist *I. ricinus* der Hauptvektor der im 3. Kapitel untersuchten Pathogene, *Babesia* spp. Diese Erreger besitzen ein zoonotisches Potential (Homer et al. 2000; Blaschitz et al. 2008; Dobler et al. 2014; Schötta et al. 2017). Koinfektionen sind in *I. ricinus* weit verbreitet und betreffen auch den Menschen (Heyman et al. 2010; Lommano et al. 2012; Chmielewska-Badora et al. 2012; Pańczuk et al. 2016). *Babesia* spp., kommen in Österreich in Wildtieren vor (Silaghi et al. 2011; Cézanne et al. 2017).

Angenommen wird auch, dass nicht nur der Zeckenstich allein zu einer Infektion führen kann, sondern auch die Handhabung und Verarbeitung von erlegtem Wild einen potentiellen Weg darstellt, sich zu infizieren (Skotarczak et al. 2008).

Wenn durch Zecken übertragene Pathogene zu einer humanen Erkrankung führen, sind multimodale Behandlungen in Betracht zu ziehen. Protozoen und bakterielle Erreger erfordern unterschiedliche Therapien (Kletsova et al. 2017; Sanchez et al. 2016). In Österreich gibt es circa 50.000 Fälle von Lyme Borreliose pro Jahr (Stanek 2009) und praktizierende Ärzte wissen darüber Bescheid. Im Gegensatz dazu hinkt das Wissen und die Forschung bei Protozoen, insbesonders bei *Babesia* spp in Österreich stark nach (Sonnleitner et al. 2014). *Babesien* spp. sind Hämoprotozoen (Schnittger et al. 2012; Hunfeld et al. 2008; Carvalho et al. 2017) d. h. sie befallen ausschließlich Erythrocyten. *B. divergens, B. veatorum, B. microti* z.B. können die menschliche Babesiose verursachen (Vannier et al. 2012). Der Schweregrad der menschlichen Babesiose reicht von asymtomatischer, moderater bis zur selbstlimitierenden fieberhaften Erkrankung. Besonders gefährlich sind diese Blutparasiten für immungeschwächte und splenektomierte Personen, bei denen eine Infektion tödlich verlaufen kann (Krause et al. 2003, Leiby 2011, Mareedu et al. 2017). Eine besondere Gefahr birgt die Übertragung durch Blutprodukte, die leider sehr unterschätzt wird, jedoch einen steigenden Trend zeigt (Bloch et al. 2016; Levin et al. 2016).

Nachweis und Verweildauer einer frischen Infektion im Menschen ist je nach Gattung verschieden (Krause et al. 1998; Mørch et al. 2015; Häselbarth et al. 2007; Herwaldt et al. 2003). Nachweis von *Babesia*.spp DNA mittels PCR ist bei einer zirkulierenden Parasitämie möglich, jedoch durch große Selbstlimitierung schwierig, besonders in einer Zeit mit niedriger Zeckenaktivität (Hunfeld et al. 2008; Leiby 2011; Vannier et al. 2012). Hingegen zeigen Untersuchungen, dass Parasitämien bei Blutspendern auch außerhalb der Hauptzeckenaktivität nachgewiesen wurden (Leiby et al. 2005) oder eine langanhaltende Parasitämie aufweisen (Moritz et al. 2016; Bloch et al. 2016).

Babesia-Infektionen können sogar nach längerer Ruhezeit auftreten (Krause et al. 1998).

Alle diese Studien beziehen sich auf den Erreger *B. microti*, der in den USA autochthon vorkommt und eine enge Affinität zu Theilerien aufweist (Homer et al. 2000). In Europa handelt es sich fast ausschließlich um Infektionen mit *B. divergens* und verwandten Arten. Bis heute gibt es in Österreich eine einzige autochthone Babesien Infektion bei einem

immunsupprimierten Menschen. Diese Babesienart ist zu *B. divergens* nahe verwandt und wurde als *Babesia* sp. EU1 indentifiziert. Derzeit ist sie unter *B*. *venatorum* bekannt (Herwaldt et al. 2003). Dieser Patient ist Jäger und hielt sich oft in freier Natur auf. Er berichtete vor Auftritt der Symptome von einem Zeckenstich. Über serologische Nachweise von *Babesia* spp. (*B*. *divergens*, *B microti*) bei Blutspendern berichtet eine Studie aus Österreich 2014 (Sonnleitner et al. 2014).

Zielsetzungen und Fragestellung der Doktorarbeit

Untersuchungen von Zoonoseerregern können präventiv zur Vermeidung von Erkrankungen beitragen, Informationen über Risikogebiete liefern und das Bewusstsein in der Öffentlichkeit über bislang noch wenig bekannte Zoonoseerreger sensibilisieren. Zoonosen sind eine erhebliche Bedrohung für die öffentliche Gesundheit. An den Schnittstellen von Wildtierhabitaten und menschlichen Aktivitäten kann eine Vielzahl von Pathogenen von Wildtieren auf Menschen und auf Haustiere übertragen werden.
Das Ziel des Dissertationsprojektes ist, Zoonoseerreger in Österreich zu untersuchen, um einen Beitrag für das Gesundheits- und Risikomanagement zu erbringen und neue Informationen für Ärzte und für die breite Bevölkerung bereitzustellen.

Fragestellung

1. Ist die Risikogruppe “JägerInnen” wirklich gefährdeter als Nichtjäger an einer AE zu erkranken?
2. Kann man anhand der Indikatorspezies Rehwild (*Capreolus capreolus*) ein FSME-Aufkommen außerhalb der schon bestehenden Risikokarte feststellen?
3. Existieren durch Vektoren übertragene wenig bekannte Pathogene in Österreich?

Die wichtigsten Untersuchungen bei den eingereichten Publikationen (Manuskript 1-3):

1. Blutuntersuchung auf den Erreger *Echinokokkus multilocularis* bei aktiven JägerInnen (1. Kapitel).
2. Analyse von Rehwildseren auf anti FSME Antikörper (2. Kapitel).
3. Blutuntersuchungen von durch Vektoren übertragenen Pathogene (*Babesia* spp). bei immunkompetenten Personen (3. Kapitel)

Aufbau der Rahmenschrift

In der Einleitung wird ein Überblick der in den Manuskripten (1-3) untersuchten zoonotischen Pathogene angeführt. Entsprechend werden im nächsten Punkt die Ziele und die Zielsetzungen dieser Arbeit genannt. Anschließend erfolgt die Vorstellung der zur Dissertation eingereichten Publikationen. Für jede Arbeit (1-3) wird zuerst der Abstract der Arbeit - auf Deutsch übersetzt - angeführt, gefolgt von den wichtigsten Ergebnissen, das Original Manuskript ist nachfolgend gelistet. Schließlich erfolgt eine Zusammenfassung der

eingereichten Publikationen und der Abschluss dieser Rahmenschrift befasst sich mit dem Ausblick und dem künftigen Forschungsbedarf.

Zoonotische Pathogene der zur Dissertation eingereichten Publikationen 1-3

1. Kapitel - Hunting poses only a low risk for alveolar echinococcosis

(Wetscher et al., 2019)

Autoren: Monika Wetscher, Klaus Hackländer, Viktoria Faber, Ninon Taylor, Herbert Auer, Georg Gerhard Duscher

Journal: Frontiers in Public Health

Kurzfassung

Das österreichische Bundesland Tirol gehört zu den Gebieten, in denen die alveoläre Echinokokkose (AE) - verursacht durch den Fuchsbandwurm, *Echinococcus multilocularis* (*E. multilocularis*) - stark endemisch ist. In Österreich wurde seit 2011 eine zunehmende Inzidenz humaner AE-Fälle beobachtet, vermutlich in Verbindung mit der zunehmenden Fuchspopulation, die mit dem Fuchsbandwurm *E. multilocularis* infiziert ist. In den Jahren 2015 und 2016 wurden 813 Seren aktiver JägerInnen in allen neun Bezirken Tirols serologisch auf *E. multilocularis* Antikörper untersucht. Einundzwanzig (2,58%) positive Ergebnisse im ELISA wurden festgestellt. Im Western blot (WB) zeigte nur ein einziges (0,12%) Serum eine geringe positive Reaktion. Bei diesem Probanden konnte bislang mittels Bildgebung (Ultraschall) keine Läsion im Leberparenchym nachgewiesen werden. Das Risiko, eine alveoläre Echinokokkose zu entwickeln, bleibt für diesen WB-positiven Jäger aber bestehen und bedarf regelmäßiger Kontrollen. Die Risikofaktoranalyse dieser 813 Jäger ergab, dass 697 (85,7%) regelmäßig Rotfüchse gejagt und 332 (40,8%) diese auch selbstständig abgebalgt haben. Dreihundertachtzehn (39,1%) der 813 Jäger waren Besitzer von Jagdhunden; 89 (10,9%) und 243 (29,9%) waren Besitzer von nichtjagenden Hunden bzw. Katzen.
Unsere Ergebnisse zeigen, dass JägerInnen im Vergleich zu Nichtjägern kein erhöhtes Risiko haben an einer alveolären Echinokokkose zu erkranken.

Wichtigste Ergebnisse der Untersuchung

Zusammenfassend zeigen die Ergebnisse, dass JägerInnen mit *E. multilocularis* in Kontakt kommen. Sie sind aufgrund der Fuchsjagd und des Abbalgens der Füchse sowie ihrer lang anhaltenden Aktivität im Freien in potenziell kontaminierten Gebieten vermehrt *E. multilocularis* ausgesetzt. Trotzdem lieferte bei der relativ großen Stichprobengröße nur ein einziger Jäger positive Ergebnisse in der Serologie, aber ohne Ultraschallbestätigung.

Bei den österreichischen AE Patienten können wir derzeit keine Präferenz zu den JägerInnen beobachten.

Erkenntnis zur Fragestellung

In unserer Kohorte konnten wir kein vermehrtes Risiko bei der untersuchten exponierten Personengruppe der JägerInnen an einer AE zu erkranken feststellen. Weitere Untersuchungen sind erforderlich um diese widersprüchlichen Ergebnisse aufzuklären und die tatsächlichen Risikofaktoren für AE zu bestimmen. Diese Studie präsentiert uns auch die Notwendigkeit die Bevölkerung über diesen Erreger genau zu informieren.
In Österreich bestehen AE Erkrankungsfälle in unterschiedlicher Schwere, z.T. auch mit letalem Ausgang. Deshalb ist die breite Aufklärung der gesamten Bevölkerung von besonderer Wichtigkeit und Bedeutung.

Original Manuskript

Front Public Health. 2019; 7: 7.
Published online 2019 Jan 29.doi:10.3389/fpubh.2019.00007

PMCID:PMC6361863
PMID:30761283

Hunting Poses Only a Low Risk for Alveolar Echinococcosis

Monika Wetscher,[1] Klaus Hackländer,[1] Viktoria Faber,[2] Ninon Taylor,[2,†] Herbert Auer,[3] and Georg G. Duscher[4,*]
Author information Article notes Copyright and License information Disclaimer

Abstract

The Austrian province of Tyrol belongs to the areas where the alveolar echinococcosis (AE) caused by the fox tapeworm *Echinococcus multilocularis* (*E. multilocularis*) is highly endemic. In Central Europe and since 2011 in Austria, a growing incidence of human cases of AE has been observed, presumably linked with increasing fox populations infected by the fox tapeworm *E. multilocularis*. Hunting and the related activities put hunters in a high-risk group, and they are considered particularly vulnerable for the contraction of an AE. In light of this risk and the increased number of AE cases made public in Austria, the objective of the study was to investigate the prevalence of AE in hunters and to provide a possible connection to the incidence increase. In 2015 and 2016, we examined 813 serums of active hunters from all nine districts of Tyrol and serologically tested them for *E. multilocularis* antibodies. Twenty-one (2.58%) positive results in ELISA were detected via Western blot (WB), and only one (0.12%)

serum showed a low positive reaction. No lesion in the liver parenchyma could be detected by abdominal ultrasonography in this patient so far, but the risk of developing alveolar echinococcosis remains for this WB-positive hunter. Risk factor analysis of these 813 hunters revealed that 697 (85.7%) hunted red foxes regularly and 332 (40.8%) of those skinned them as well. Three hundred and eighteen (39.1%) out of the 813 hunters were owners of hunting dogs; 89 (10.9%) and 243 (29.9%) were owners of non-hunting dogs and cats, respectively. Our results indicate that hunters do not have a greater risk of infection with *E. multilocularis* compared to non-hunters in Austria. The cause of the unexpected increase in AE cases in Austria remains unclear.

Keywords: *Echinococcus multilocularis*, active hunters, serological screening, risk factor, Austria

Introduction

Human alveolar echinococcosis (AE) is caused by the metacestode stage of the fox tapeworm *Echinococcus multilocularis* (1). In Central Europe, this rare parasitic disease that is potentially fatal in humans (2), primarily circulates within a sylvatic life cycle comprising red foxes (*Vulpes vulpes*) as definitive hosts and rodents (e.g., *Microtus arvalis, Arvicola terrestris, Ondatra zibethica*) as intermediate hosts (3, 4). The raccoon dog (*Nyctereutes procyonoides*) as neozoon species plays a similar role as the red fox as final host (4, 5). Also, pets, especially dogs, can also act as final hosts and contaminate the environment by excreting infective *E. multilocularis* eggs (6–10). Humans acquire the infection by peroral ingestion of *E. multilocularis* eggs present in contaminated soil, food, or animal skins (6, 11, 12). Humans acquire the infection by peroral ingestion of *E. multilocularis* eggs present in contaminated soil, food, or animal skins (6, 11, 12). The *E. multilocularis* metacestode in the intermediate host establishes itself primarily in the liver and shows a tumor-like growth pattern similar to a malignant tumor (13–15). Due to the prolonged growth of the parasite, the lack of specific symptoms in many cases, and the long incubation period of up to 20 years, AE is often diagnosed and treated in a rather late and metastatic stage (11, 16). Serological tests for detection of AE are available and allow early detection of disease before clinical manifestation (1, 17). During recent decades the fox population increased due to successful rabies vaccination (18, 19). Hunting has repeatedly been published as a risk factor (20–24) and associated with AE acquisition (25). Further, there has been an increasing incidence of human AE cases in Austria, especially in the western provinces of Austria (Tyrol and Vorarlberg) (23). These increased AE cases were unexpected. Thus, it was recommended to improve the surveillance system in Austria by screening exposed individuals such as hunters, especially in *E. multilocularis*-endemic regions, to detect early AE cases (23).To obtain data on the current situation regarding the aforementioned epidemiological development, we performed a

serological study on anti-*E. multilocularis* antibodies comprising hunters of all nine Tyrolean districts in 2015 and 2016. This particular group was selected because of their potentially high exposure to eggs from infested foxes through their hunting activity, with the aim of quantifying human AE prevalence in hunters.

Materials and Methods

Study Area

The study area is the province of Tyrol with its nine districts. Tyrol is bordered by the provinces of Salzburg to the east, Vorarlberg to the west, and Carinthia to the south. The neighboring countries of Tyrol are Germany (to the north), Italy (to the south), and Switzerland (to the west).

Sample Collection

The Hunting Association of Tyrol invited their members to participate at the annual meetings for the display of trophies in the capital cities of their districts. Out of 16,146 active hunters, 813 (i.e., 5%, 736 males and 77 females) were willing in 2015 and 2016 to provide blood samples at these events.

Approval for this study was obtained from the Ethics Committee in Salzburg on January 28th, 2015 (No: 415-E/1845/2-2015). The written informed consent was given by the patients for their information to be stored and used for this study.

For demographic data and risk factors, each participant was also asked to complete a questionnaire concerning their hunting activity. The issues raised were temporal hunting activity, killing of red foxes, skinning of foxes as well as hunting dog ownership. Further questions concerned the ownership of pets such as dogs or cats.

The venous blood samples taken by medical doctors were performed using Vacuette®–Multiple Use Drawing Needle (Greiner Bio-One GmbH. 4,550 Kremsmünster, Austria) and Vacuette®–8 ml Z Serum Sep Clot Activator (Greiner Bio-One GmbH. 4,550 Kremsmünster, Austria). The samples were stored at +5°C.

Serum tubes and questionnaires were numbered and checked twice by independent persons (medical doctors and a principal investigator) for their reliability.

Serological Testing

The analysis of the sera for anti-*E. multilocularis* antibodies was performed within 48 h at the Specific Prophylaxis and Tropical Medicine (SPTM). As a basic test, an enzyme-linked immunosorbent assay (ELISA) with *E. multilocularis* crude antigen was used (26). A

commercial western blot (WB, LDBio, France) was used to confirm the positive results (27). Serum samples were considered serologically positive if they showed clearly positive reactions in the ELISA (cut-off: 20 Antibody Units based on a positive control serum with 100 AUs). Persons with positive antibody values were examined by WB and were informed by the principal investigator. In addition, they were asked to provide a further blood sample, and in the case of verification of positive test results, the participants were again personally contacted and ultrasound/computed tomography of the liver was recommended.

Data Analysis

All available details about age, hunting activities, pet ownership, and serological test were logged into an Excel spreadsheet (Microsoft Office Excel, Redmond, USA) and analyzed.

Results

In total, 813 (736 males and 77 females) sera were evaluated during the 2 years human survey. The mean age of the hunters was 52.5 years and ranged from 18 to 86. The age distribution was as follows: 4.7% were 18–25 years old, 23.1% were 26–45 years old, 44% were 46–60 years old, and 28.2% were older than 60 years of age. All of the participants had been hunting for, at least, 1 year, 26.6% for 2–10 years and 20.8% for 11–20 years, whereas 52.6% had been actively hunting for more than 20 years.

Out of 813 hunters, 697 (85.7%) were regularly hunting red foxes, and 332 (40.8%) of these also skinned foxes. About 318 (39.1%) of the hunters had currently or in the recent past a hunting dog, and 89 (10.9%) hunters owned a non-hunting dog. Cat ownership was reported among 243 (29.9%) of the hunters (Table 1).

Table 1: Number of examined hunters in the nine Tyrolean districts, their hunting activities and pet ownership							
	n (%)	n (%)	n (%)	n (%)		n (%) ownership of pet	
district	examined hunters	shoot foxes	skinning foxes	hunting dog owner	active hunting period [a]	dog	cat	or both
Innsbruck	37 (4,6)	28 (4,0)	13 (3,9)	12 (3,8)	17	6 (6,7)	8 (3,3)	3 (2,5)
Innsbruck-Land	98 (12,1)	84 (12,1)	30 (9,0)	32 (10,1)	21	8 (9,0)	27 (11,1)	11 (9,2)
Imst	43 (5,3)	41 (5,9)	20 (6,0)	20 (6,3)	29	2 (2,2)	15 (6,2)	10 (8,4)
Kitzbühel	98 (12,1)	92 (13,2)	49 (14,8)	32 (10,1)	26	16 (18,0)	31 (12,8)	13 (10,9)
Kufstein	123 (15,1)	111 (15,9)	56 (16,9)	47 (14,8)	25	11 (12,4)	38 (15,6)	18 (15,1)
Landeck	110 (13,5)	88 (12,6)	36 (10,8)	45 (14,2)	22	3 (3,4)	21 (8,6)	8 (6,7)
Lienz	119 (14,6)	98 (14,1)	38 (11,4)	52 (16,4)	22	18 (20,2)	42 (17,3)	26 (21,8)
Reutte	114 (14,0)	93 (13,3)	47 (14,2)	44 (13,8)	22	21 (23,6)	40 (16,5)	22 (18,5)
Schwaz	71 (8,7)	62 (8,9)	43 (13,0)	34 (10,7)	25	4 (4,5)	21 (8,6)	8 (6,7)
total	813 (100,0)	697 (85,7)	332 (40,8)	318 (39,1)	23	89 (10,9)	243 (29,9)	119 (14,6)

[a]Average of the active hunting period in years.

The serological analysis for antibodies against *E. multilocularis* antigen showed a low positive ELISA result in 21 (2.6% participants) (Table 2 shows the hunting activities of the positive ELISA hunters), and one serum was positive by both ELISA and WB (Table 3). This serum originated from a male hunter from the district of Lienz (seroprevalence: 0.12%), and the abdominal sonography showed no lesion changes in the liver parenchyma. This 80 years-old hunter was hunting for 62 years, shot and skinned foxes regularly, and had neither a hunting dog nor pets regularly. The real prevalence among the hunters in this cohort is 0 due to the lack of any confirmed AE case.

Table 2: List of the 20 EmELISA positive hunters and their hunting activities and pet ownership. Except the hunter with ELISA and WB positive findings, described separately.

n EmELISA positive	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	∑
shoot foxes	X	X	X	X	X	X	X	X	/	X	X	X	X	X	X	X	X	X	X	X	19
skinning foxes	X	X	X	/	X	/	/	/	/	X	/	X	/	X	X	/	/	X	/	/	9
hunting dog owner	X	/	/	/	X	/	X	/	/	/	X	X	/	X	X	X	X	X	/	/	10
dog						X										/	/	/	/	/	1
cat				X		X	X	X		X	X		X			/	/	/	/	/	7

Table 3: Number of licensed and examined hunters in the nine Tyrolean districts and the serological test results

district	[a]n of hunters	n (%) of hunters examined	n (%) of hunters ELISA positive	n (%) of hunters WB positive
Innsbruck	599	37 (4,6)	2	0
Innsbruck-Land	2.985	98 (12,1)	1	0
Imst	1.880	43 (5,3)	3	0
Kitzbühel	1.499	98 (12,1)	2	0
Kufstein	1.582	123 (15,1)	7	0
Landeck	1.988	110 (13,5)	1	0
Lienz	2.006	119 (14,6)	4	1
Reutte	1.262	114 (14,0)	0	0
Schwaz	2.345	71 (8,7)	1	0
total	16.146	813 (100,0)	21 (2,58)	1 (0,12)

[a]Licensed hunters per district

One of the 813 Tyrolean hunters (district Kitzbühel) had previously been treated successfully by surgical intervention (in-toto-extirpation of the parasite) in 2011. In our screening study, however, he presented a negative test result.

Discussion

In the years 2015 and 2016, we tested the sera of 813 hunters from the province of Tyrol to evaluate the current situation of AE in the presumed risk group “hunters” originating from a highly endemic area (1, 7, 13). However, AE is still a life-threatening disease for humans (11), but the prognosis is improved if the infection is diagnosed at a very early stage (even long before clinical manifestation). The serum sample of an 80 years-old hunter from the district of Lienz yielded a positive result by ELISA and by WB. In this case an infection with AE can be assumed and further confirmed by using ultrasound as the imaging screening method of choice (28). So far no visible lesions in the liver of this patient could be found. Thus, the hunter was instructed to repeat serological tests as well as ultrasound examination twice a year during the following years. In effect, liver lesions can take years to become apparent in seropositive persons (29), and lesions <2 cm are difficult to diagnose (30). Due to its higher accuracy in detecting liver lesions [although without complete proof (31)], computerized tomography could be applied and was suggested but not carried out in this case, as this is the responsibility of the general practitioner.

Also, among another 20 hunters from all other districts (except in Reutte), low positive antibody levels could be determined, which could not be confirmed by WB. In these cases, it is necessary to carry out follow-up investigations to exclude an early stage of infection (1, 32), at least, once a year during the next 2 years (17, 29). Another explanation for this finding could

be a cross-reaction in the ELISA (33). We can exclude *E. granulosus* in all 20 ELISA and WB positive *E. multilocularis* results by testing with indirect haemagglutination test (IHA) (26).

Although 792 hunters (97.4%) were serologically negative, documenting that they were not infected currently, all were strongly encouraged to re-examine their *Echinococcus*-immune status individually in intervals of 2–3 years, at least, during their active hunting period. The observation of seroconversion from negative to positive would demonstrate that a fresh infection must have occurred since the last examination.

If particularly exposed persons are regularly monitored by ultrasound imaging or by serological screenings (1, 34), the development of the slow-growing metacestode is rather limited and a cure becomes more likely. Hunters were considered as one of these risk groups (20–25) based on regular contact with foxes and pets (6, 35) as well as with contaminated fur (12) and environment.

In this study, 86% of hunters reported shooting foxes and 41% admitted to skinning foxes regularly. Also, 39% keep hunting dogs and up to 30% are owners of dogs or cats as pets. Despite these high-hazard exposure potentials due to long hunting activities, as almost 52.6% had been hunting for more than 20 years, there was no clear evidence to make a diagnosis of AE. Similar to a study of trappers with high hunting activity in South Dakota, none of the trappers showed antibody evidence for the presence of *E. multilocularis* (36). In a case-control study from Tyrol, hunting was published as an independent risk factor in relation to AE (25). In contrast, (7) were not able to prove a connection between hunting and AE with double the number of test subjects in neighboring Germany. Different methods give differentiated statements about the risk of hunting; case-control studies increase the probability that hunting is a risk factor, but not significantly. This was shown in a recently published study in which case-control and cross-sectional studies were compared and no connection between hunting and AE Echinococcosis was proven (37). In south Gansu, China, a large number of AE cases was diagnosed using serological screening and ultrasonography, and hunting had no effect as a risk factor (38). A total of 23,321 people were examined in three published serological screening studies in Austria (21, 39, 40). Two AE cases could be diagnosed with this preventive screening examination; both cases occurred in the province of Tyrol and had no connection to hunting. Given these results, we assume that hunters are not at a higher risk of contracting an AE than non-hunters. Furthermore, the increase in confirmed AE cases in Austria could not be ascribed to hunting due to a lack of available data on occupation and leisure activities (23). The study of 149 (100%) hunters in south-eastern Austria showed 7 (5%) subjects with a positive ELISA value and none could be confirmed in WB (21). In our studies, with a 5.5 times higher number of test subjects, there were 2.6 and 0.12%, ELISA and WB, respectively, positive results. The ELISA reactions show that increased contact with *E.*

multilocularis eggs (41) takes place due to hunting. In healthy individuals, however, only a low level of manifestation takes place and an AE is prevented (42). In contrast, a weakened immune system promotes infection of *E. multilocularis* in humans (43, 44) and significantly increases the risk of contracting AE (45, 46). In the course of this study, we tested one individual whose AE liver lesion was surgically removed in 2011. The lack of antibodies in this subject is presumably due to the success of the surgery (47). Furthermore, it should be mentioned that this test subject suffered from cancer and had immunosuppressive therapy beforehand.

Studies show that pets, dogs, and cats excrete infectious *E. multilocularis* eggs (6, 8–10). The case-control study from Tyrol, in which hunting poses a risk, also showed a connection between cats and AE in western Austria (25). In this single case-control study in Austria, dog ownership was reported as a low-risk factor. In contrast, another case-control study showed dog ownership, especially where the dogs spend a lot of time outdoors, as a major risk factor (7). An increase in human seroprevalence was reported in an area with a high prevalence of *E. multilocularis* in intermediate hosts (rodents) and prevalence detection of *E. multilocularis* in domestic animals, however, without an increase in human AE cases (48). In our study, 69% of test subjects had a hunting dog as well as dogs and cats as pets. The potential risk of infection is unknown in Austria, as there is no data on the prevalence of *E. multilocularis* in dogs and cats. Our study shows that despite considerable possession of hunting dogs and pets, no AE was diagnosed in the risk group of hunters.

To summarize, the results indicate that hunters come into contact with *E. multilocularis*, which was ascertained by positive ELISA and WB tests. However, thus far, we have not been able to observe that the proportion of hunters is higher than the proportion of non-hunters among all Austrian AE patients. The unexpected AE cases in Austria could not be explained with our investigations of an exposed group of “hunters.” This study demonstrates the need for further research to elucidate on conflicting results and identify the actual risk factors for AE to be able to avoid preventable AE illnesses. Missing data, especially in pets about the prevalence rates of *E. multilocularis* in Austria, should be investigated in future.

Ethics Statement

This study was carried out in accordance with the recommendations of the Ethics Committee in Salzburg. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee in Salzburg on January 28th, 2015. 415-E/1845/2-2015.

Author Contributions

MW designed the study. MW and VF were responsible for taking blood samples. MW, KH, HA, VF, NT, and GD all contributed to the manuscript editing and approved the final version of this manuscript.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

We thank the Tyrolean hunting association (Tiroler Jägerverband) for financial support to conduct this study. We also appreciate all the Tyrolean hunters for their participation in this project. Also, we wish to thank Ina Kandil, Walburga Maderthaner, Sabine Rosenlechner, Michael Rosenlechner, and Michaela Schachner.

References

1. Auer H. Die Bedeutung labordiagnostischer Untersuchungen für die Klinik, die Epidemiologie und Prävention der alveolären Echinokokkose – Erfahrungen zweier Jahrzehnte in Österreich. [Relevance of parasitological examinations for the clinical course, epidemiology and prevention of alveolar echinococcosis – experiences of more than two decades in Austria]. *Wien. Klin. Wochenschr.* (2006) 118 (Suppl 3):18–26. doi: 10.1007/s00508-006-0673-3

CrossRef Full Text | Google Scholar

2. Piarroux M, Piarroux R, Giorgi R, Knapp J, Bardonnet K, Sudre B, et al. Clinical features and evolution of alveolar echinococcosis in France from 1982 to 2007: results of a survey in 387 patients. *J Hepatol.* (2011) 55:1025–33. doi: 10.1016/j.jhep.2011.02.018

PubMed Abstract | CrossRef Full Text | Google Scholar

3. Eckert J, Deplazes P, Craig PS, Gemmell MA, Gottstein B, Heath D, et al. Echinococcosis in animals: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski Z, editors. *WHO/OIE Manual on Echinococcosis in Humans and Animals.* Courtesy of the Institute of Parasitology, University of Zurich (2001). p. 73–99.

4. Oksanen A, Siles-Lucas M, Karamon J, Possenti A, Conraths FJ, Romig T, et al. The geographical distribution and prevalence of *Echinococcus multilocularis* in animals in the

European Union and adjacent countries: a systematic review and meta-analysis. *Parasit Vectors* (2016) 9:519. doi: 10.1186/s13071-016-1746-4

PubMed Abstract | CrossRef Full Text | Google Scholar

5. Duscher T, HodŽić A, Glawischnig W, Duscher GG. The raccoon dog (*Nyctereutes procyonoides*) and the raccoon (*Procyon lotor*) – their role and impact of maintaining and transmitting zoonotic diseases in Austria, Central Europe. *Parasitol Res.* (2017) 116:1411–1416. doi: 10.1007/s00436-017-5405-2

PubMed Abstract | CrossRef Full Text | Google Scholar

6. Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *J Clin Microbiol Rev.* (2004) 17:107–35. doi: 10.1128/CMR.17.1.107-135.2004

PubMed Abstract | CrossRef Full Text | Google Scholar

7. Kern P, Ammon A, Kron M, Sinn G, Sander S, Petersen LR, et al. Risk factors for alveolar echinococcosis in humans. *Emerg Infect Dis.* (2004) 10:2088–93. doi: 10.3201/eid1012.030773

PubMed Abstract | CrossRef Full Text | Google Scholar

8. Duscher GG, Leschnik M, Fuehrer HP, Joachim A. Wildlife reservoirs for vector-borne canine, feline and zoonotic infections in Austria. *Int J Parasitol Parasit Wildl.* (2015) 4:88–96. doi: 10.1016/j.ijppaw.2014.12.001

PubMed Abstract | CrossRef Full Text | Google Scholar

9. Karamon J, Samorek-Pieróg M, Kochanowski M, Dabrowska J, Sroka J, Gołab E, et al. First detection of *Echinococcus multilocularis* in dogs in a highly endemic area of Poland. *Folia Parasitol.* (2016) 63:2016.018. doi: 10.14411/fp.2016.018

PubMed Abstract | CrossRef Full Text | Google Scholar

10. Bastien M, Vaniscotte A, Combes B, Umhang G, Germain E, Gouley V, et al. High density of fox and cat faeces in kitchen gardens and resulting rodent exposure to *Echinococcus multilocularis* and *Toxoplasma gondii*. *Folia Parasitol.* (2018) 65:2018.002. doi: 10.14411/fp.2018.002

PubMed Abstract | CrossRef Full Text | Google Scholar

11. Brunetti E, Kern P, Vuitton DA, Writing Panel for the WHO.-IWGE. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop.* (2010) 114:1–16. doi: 10.1016/j.actatropica.2009.11.001

CrossRef Full Text | Google Scholar

12. Nagy A, Ziadniov I, Schweiger A, Schnyder M, Deplazes P. Fellkontamination mit Eiern von zoonotischen Helminthen bei Hof- und Haushunden sowie bei Füchsen. [Hair coat contamination with zoonotic helminth eggs of farm and pet dogs and foxes]. *Berl Munch Tierarztl Wschr.* (2011) 124:503–11.

13. Kern P, Bardonnet K, Renner E, Auer H, Pawlowski Z, Ammann RW, et al. European echinococcosis registry: human alveolar echinococcosis, Europe, 1982-2000. *Emerg Infect Dis.* (2003) 9:343–9. doi: 10.3201/eid0903.020341

PubMed Abstract | CrossRef Full Text | Google Scholar

14. Tsaroucha AK, Polychronidis AC, Lyrantzopoulos N, Pitiakoudis MS, Karayiannakis AJ, Manolas KJ, et al. Hydatid disease of the abdomen and other locations. *World J Surg.* (2005) 29:1161–5. doi: 10.1007/s00268-005-7775-3

PubMed Abstract | CrossRef Full Text | Google Scholar

15. Ricken FJ, Nell J, Grüner B, Schmidberger J, Kaltenbach T, Kratzer W, et al. Albendazole increase the inflammatory response and the amount of Em2-positive small particles of *Echinococcus multilocularis* (spems) in human hepatic alveolar echinococcosis lesions. *PLoS Negl Trop Dis.* (2017) 11:e0005636. doi: 10.1371/journal.pntd.0005636

CrossRef Full Text | Google Scholar

16. Goetz M, Hainz M, Schadmand-Fischer S, Kittner JM, Heinrich S. Echinococcosis mimicking autoimmune or malignant bile duct disease. *Gastroenterol* (2013) 51:1089–91. doi: 10.1055/s-0033-1335751

CrossRef Full Text | Google Scholar

17. Bartholomot B, Vuittin AD, Harraga S, Shi DZ, Giraudoux P, Barnish G, et al. Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. *Am J Trop Med Hyg.* (2002) 66:23–9. doi: 10.4269/ajtmh.2002.66.23

PubMed Abstract | CrossRef Full Text | Google Scholar

18. Duscher G, Pleydell D, Pros LH, Joachim A. *Echinococcus multilocularis* in Austrian foxes from 1991 until 2004. *J Vet Med B* (2006) 53:138–44. doi: 10.1111/j.1439-0450.2006.00930.x

PubMed Abstract | CrossRef Full Text | Google Scholar

19. Schweiger A, Ammann RW, Candinas D, Clavien PA, Eckert J, Gottstein B, et al. Human alveolar echinococcosis after fox population increase, Switzerland. *Emerg Infect Dis.* (2007) 13:878–82. doi: 10.3201/eid1306.061074

PubMed Abstract | CrossRef Full Text | Google Scholar

20. Eckert J. *Echinococcus multilocularis* in Europa – Versuch einer Risikobewertung. [Echinococcus multilocularis in Europe: an attempt to assess the risk]. *Mitt Österr Ges Tropenmed Parasitol.* (1996) 18:131–40.

Google Scholar

21. Deutz A, Fuchs K, Schuller W, Nowotny N, Auer H, Aspöck H, et al. Seroepidemiologische Untersuchung von Jägern in Südostösterreich – Prävalenzen, Risikopotentiale und Vorbeugemaßnahmen [Seroepidemiological studies of zoonotic infections in hunters in southeastern Austria – prevalences, risk factors and preventive methods]. *Berl Munch Tierarztl Wschr.* (2003) 116:306–11.

Google Scholar

22. Tiaoying L, Jiamin Q, Wen Y, Craig PS, Xingwang C, Ning X, et al. Echinococcosis in Tibetan populations, Western Sichuan, province, China. *Emerg Infect Dis.* (2005) 11:1866–73. doi: 10.3201/eid1112.050079

PubMed Abstract | CrossRef Full Text | Google Scholar

23. Schneider R, Aspöck H, Auer H. Unexpected increase of alveolar echinococcosis, Austria, 2011. *Emerg Infect Dis.* (2013) 19:475–7. doi: 10.3201/eid1903.120595

CrossRef Full Text | Google Scholar

24. Sadkowska-Todys M, Baumann-Popczyk A, Wnukowska N, Popczyk B, Kucharczyk B, Golab E. Occurrence and prevalence of selected zoonotic agents: *echinococcus multilocularis, Trichinella spiralis* and hepatitis E virus (HEV) in the population of Polish hunters – results of the study conducted in 2010-2012. *Przegl Epidemiol.* (2015) 69:673–8.

PubMed Abstract | Google Scholar

25. Kreidl P, Allerberger F, Judmaier G, Auer H, Aspöck H, Hall AJ. Domestic pets as risk factors for alveolar hydatid disease in Austria. *Am J Epidemiol.* (1998) 147:978–81.

PubMed Abstract | Google Scholar

26. Auer H, Picher O, Aspöck H. Combined application of enzyme-linked immunosorbent assay (ELISA) and indirect haemagglutination test (IHA) as a useful tool for the diagnosis and post-operative surveillance of human alveolar and cystic echinococcosis. *Zbl Bakt Hyg A* (1988) 270:313–25.

PubMed Abstract | Google Scholar

27. Liance M, Janin V, Bresson-Hadni S, Vuitton DA, Houin R, Piarroux R. Immunodiagnosis of Echinococcus infections: confirmatory testing and species differentiation by a new commercial western blot. *J Clin Microbiol.* (2000) 38:3718–21.

PubMed Abstract | Google Scholar

28. Mihmanli M, Idiz UO, Kaya C, Demir U, Bostanci O, Omeroglu S, et al. Current status of diagnosis and treatment of hepatic echinococcosis: *World J Hepatol.* (2016) 8:1169–81. doi: 10.4254/wjh.v8.i28.1169

PubMed Abstract | CrossRef Full Text | Google Scholar

29. Jensen B, Reuter S, Kratzer W, Naser K, Kächele V, Kimmig P, et al. Long term seropositivity against *Echinococcus multilocularis* in an epidemiological follow-up study in Southwestern Germany (Römerstein). *Infection* (2001) 29:310–14. doi: 10.1007/s15010-001-1153-3

PubMed Abstract | CrossRef Full Text | Google Scholar

30. Liu W, Delabrousse E, Blagosklonov O, Wang J, Zeng H, Jiang Y, et al. Innovation in hepatic alveolar echinococcosis imaging: best use of old tools and necessary evaluation of new ones. *Parasite* (2014) 21:1–14. doi: 10.1051/parasite/2014072

PubMed Abstract | CrossRef Full Text | Google Scholar

31. Reuter S, Nüssle K, Kolokythas O, Haug U, Rieber A, Kern P, et al. Alveolar liver echinococcosis: a comparative study of three imaging techniques. *Infection* (2001) 29:119–25. doi: 10.1007/s15010-001-1081-2

PubMed Abstract | CrossRef Full Text | Google Scholar

32. Bresson-Hadni S, Laplante JJ, Lenys D, Rohmer P, Gottstein B, Jacquier P, et al. Seroepidemiologic screening of *Echinococcus multilocularis* infection in a European area endemic for alveolar echinococcosis. *Am J Trop Med Hyg.* (1994) 51:837–46. doi: 10.4269/ajtmh.1994.51.837

PubMed Abstract | CrossRef Full Text | Google Scholar

33. Gottstein B, Jacquier P, Bresson-Hadni S, Eckert J. Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2Plus antigen. *J Clin Microbiol.* (1993) 13:373–6.

Google Scholar

34. Romig T, Kratzer W, Kimmig P, Frosch M, Gaus W, Flegel WA, et al. An epidemiologic survey of human alveolar echinococcosis in southwestern Germany. Römerstein Study Group. *Am J Trop Med Hyg.* (1999) 61:566–73.

PubMed Abstract | Google Scholar

35. Craig PS. Epidemiology of human alveolar echinococcosis in China. *Parasitol Int.* (2006) 55:221–5. doi: 10.1016/j.parint.2005.11.034

PubMed Abstract | CrossRef Full Text | Google Scholar

36. Hildreth MB, Sriram S, Gottstein B, Wilson M, Schantz PM. Failure to identify alveolar echinococcosis in trapper from South Dakota in spite of high prevalence of *Echinococcus multilocularis* in wild canids. *J Parasitol.* (2000). 86:75–7. doi: 10.1645/0022-3395(2000)086[0075:FTIAEI]2.0.CO;2

CrossRef Full Text | Google Scholar

37. Conraths FJ, Probst C, Possenti A, Boufana B, Saulle R, La Torre G, et al. Potential risk factors associated with human alveolar echinococcosis: systematic review and meta-analysis. *PLOS Negl Trop Dis.* (2017) 11:1–15. doi: 10.1371/journal.pntd.0005801

PubMed Abstract | CrossRef Full Text | Google Scholar

38. Craig PS, Giraudoux P, Shi D, Bartholomot B, Barnish G, Delattre P, et al. An epidemiological and ecological study of human alveolar echinococcosis transmission in south Gansu, China. *Acta Trop.* (2000) 77:167–77. doi: 10.1016/S0001-706X(00)00134-0

PubMed Abstract | CrossRef Full Text | Google Scholar

39. Auer H, Schönitzer D, Judmaier G, Conrad F, Aspöck H. Seroepidemiologisches Screening als Grundlage präventivmedizinischer Maßnahmen zur Früherkennung der alveolären Echinokokkose. [Seroepidemiologic screening as a tool for the early detection of alveolar echinococcosis]. *Mitt Österr Ges Tropenmcd Parasitol.* (1995) 17:77–84.

Google Scholar

40. Poeppl W, Herkner H, Tobudic S, Faas A, Mooseder G, Burgmann H, Auer H. Exposure to *Echinococcus multilocularis, Toxocara canis*, and *Toxocara cati* in Austria: a nationwide cross-sectional seroprevalence study. *Vector Borne Zoonot Dis.* (2013) 13:798–803. doi: 10.1089/vbz.2012.1283

PubMed Abstract | CrossRef Full Text | Google Scholar

41. Beldi G, Müller N, Gottstein B. [Die alveoläre Echinokokkose]. *Swiss Med Forum* (2017) 17:760–66. doi: 10.4414/smf.2017.03046

CrossRef Full Text | Google Scholar

42. Vuitton DA, Gottstein B. *Echinococcus multilocularis* and its intermediate host: a model of parasite–host interplay. *J Biomed Biotechnol.* (2010) 2013:923193. doi: 10.1155/2010/923193

CrossRef Full Text | Google Scholar

43. Vuitton DA, Zhang SL, Yang Y, Godot V, Beurton I, Mantion G, et al. Survival strategy of *Echinococcus multilocularis* in the human host. *Parasitol Intern.* (2006) 55:51–5. doi: 10.1016/j.parint.2005.11.007

PubMed Abstract | CrossRef Full Text | Google Scholar

44. Noormahomed EV, Nhacupe N, Mascaró-Lazcano C, Mauaie MN, Buene T, Funzamo CA, et al. A cross-sectional serological study of cysticercosis, schistosomiasis, toxocariasis and echinococcosis in HIV-1 infected people in Beira, Mozambique. *PLoS Negl Trop Dis.* (2014) 8:e3121. doi: 10.1371/journal.pntd.0003121

PubMed Abstract | CrossRef Full Text | Google Scholar

45. Chauchet A, Grenouillet F, Knapp J, Richou C, Delabrousse E, Dentan C, et al. Increased incidence and characteristics of alveolar echinococcosis in patients with immunosuppression-associated conditions. *Clin Infect Dis.* (2014) 59:1095–104. doi: 10.1093/cid/ciu520

PubMed Abstract | CrossRef Full Text | Google Scholar

46. Vuitton DA, Demonmerot F, Knapp J, Richou C, Grenouillet F, Chauchet A, et al. Clinical epidemiology of human AE in Europe. *Vet Parasitol.* (2015) 213:110–20. doi: 10.1016/j.vetpar.2015.07.036

PubMed Abstract | CrossRef Full Text | Google Scholar

47. Wen H, Bresson-Hadni S, Vuitton DA, Lenys D, Yang BM, Ding ZX, et al. Analysis of immunoglobulin G subclass in the serum antibody responses of alveolar echinococcosis patients after surgical treatment and chemotherapy as an aid to assessing the outcome. *Trans R Soc Trop Med Hyg.* (1995) 89:692–7. doi: 10.1016/0035-9203(95)90449-2

PubMed Abstract | CrossRef Full Text | Google Scholar

48. Gottstein B, Saucy F, Deplazes P, Reichen J, Demierre G, Busato A, et al. Is high prevalence of *Echinococcus multilocularis* in wild and domestic animals associated with disease incidence in humans? *Emerg Infect Dis.* (2001) 7:408–12. doi: 10.3201/eid0703.017307

PubMed Abstract | CrossRef Full Text | Google Scholar

Keywords: *Echinococcus multilocularis*, active hunters, serological screening, risk factor, Austria

Citation: Wetscher M, Hackländer K, Faber V, Taylor N, Auer H and Duscher GG (2019) Hunting Poses Only a Low Risk for Alveolar Echinococcosis. *Front. Public Health* 7:7. doi: 10.3389/fpubh.2019.00007

Received: 17 January 2018; **Accepted:** 09 January 2019; **Published:** 29 January 2019.

Edited by:
Olivier Vandenberg, LHUB-ULB, Belgium
Reviewed by:
Aleksandra Barac, University of Belgrade, Serbia
Bertrand Jacques Losson, University of Liege, Belgium

Copyright © 2019 Wetscher, Hackländer, Faber, Taylor, Auer and Duscher. This is an open-access article distributed under the terms of the **Creative Commons Attribution License (CC BY)**. The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

***Correspondence:** Georg G. Duscher, **georg.duscher@vetmeduni.ac.at**

†Present Address: Ninon Taylor, Department of Dermatology, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria

2. Kapitel - Roe deer sera used for TBE surveillance in Austria

(Duscher et al., 2015)

Autoren: Georg Gerhard Duscher, Monika Wetscher, Raphaela Baumgartner, Gernot Walder

Journal: Ticks and Tick-borne Diseases

Kurzfassung

Die große Mehrheit der österreichischen Bürger ist sich der Frühsommer-Meningoenzephalitis (FSME) bewusst, dies widerspiegelt eine hohe Durchimpfungsrate von 85%. Im Gegenzug könnten die Risikobewertung und die Kartierung von Krankheiten bei Menschen durch hohe und inhomogene Impfraten und Lebensräume von Menschen behindert werden. Das Rehwild dient als Ausgangspunkt für die ganzheitliche Betrachtung des tatsächlichen FSME-Risikos in Österreich. Das Rehwild weist mehrere Merkmale auf, die es als Indikatorspezies geeignet machen. Das Rehwild ist sehr standorttreu und es ist bekannt, dass es einen starken Befall von Zecken aufweist. Darüber hinaus serokonvertiert es nach Infektionen mit FSME, aber es erkrankt nicht daran.
945 Rehwildseren wurden in ganz Österreich gewonnen und mit IFAT auf Antikörper gegen FSME gescreent. Zweiundzwanzig (2,4%) positive Proben und 17 (1,8%) Proben mit einem grenzwertigen Titer von 1:16 wurden identifiziert. Der Großteil der positiven Proben (70,6%), wurde bekannten FSME-Gebieten - basierend auf menschlichen Erkrankungsfällen - zugeordnet. Weitere Forschung ist erforderlich, um neue endemische Herde der FSME-Übertragung zu finden.

Wichtigste Ergebnisse der Untersuchung

Durch das ubiquitäre Vorkommen, die hohe Standorttreue, die Serokonversion nach Zeckenkontakt mit FSME-Viren und den Erhalt von Rehwildseren durch Zusammenarbeit mit der österreichischen Jägerschaft, die bei regulären Jagdaktivitäten gesammelt wurden, konnten wir das Rehwild als eine sehr gute Indikatorspezies darstellen.
FSME Vorkommen in Gebieten, die in der bestehenden FSME Karte von Österreich noch nicht ausgewiesen sind, konnten detektiert werden.

Erkenntnis zur Fragestellung

Das Ziel in dieser Arbeit war es neue FSME-Risikogebiete anhand von Rehwildseren zu detektieren. Unsere Untersuchungen zeigen, dass das Gefahrenpotential - auch außerhalb der ausgewiesenen Risikogebiete - gegeben ist, sich mit FSME Viren zu infizieren. Aufgrund der Tatsache, dass die bestehende FSME-Risikokarte anhand vom Wohnort der diagnostizierten Humanfälle erstellt wird und Infektions - und Wohnort möglicherweise nicht

ident sind, tragen diese Ergebnisse zur Prävention bei. Insbesondere in Gebieten mit hoher Durchimpfungsrate bei den Bewohnern, sollte diese Methode zusätzliche Informationen über die Verbreitung des Virus geben.

Original Manuskript

Ticks and Tick-borne Diseases
Volume 6, Issue 4, June 2015, Pages 489-493

Contents lists available at ScienceDirect
Ticks and Tick-borne Diseases
journal home page: w ww.elsevier.com/locate/ttbdis

Original article

Roe deer sera used for TBE surveillance in Austria

Georg GerhardDuscher[a]* MonikaWetscher[a1] RaphaelaBaumgartner[b] GernotWalder[b]

[a] Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine, Vienna, Veterinaerplatz 1, A-1210 Vienna, Austria [b] Dr. Gernot Walder GmbH, Außervillgraten, Austria

article info
Article history: Received 10 November 2014
Received in revised form 19 March 2015
Accepted 21 March 2015
Available online 11 April 2015

Abstract

A large majority of Austrian citizens are aware of tick-borne encephalitis (TBE), consequently reflected by a high vaccination rate of 85%. In return, risk assessment and disease mapping on human cases might be hampered due to high and inhomogeneous vaccination rates and travel habitats of humans. The roe deer was used to obtain a starting point for the integral view

on the actual risk of TBE in Austria. The roe deer exhibits several attributes which makes it suitable as an indicator species: the roe deer has a restricted home range and it is known to be a heavy tick carrier. Furthermore it sero-converts after infection with TBE, but no outbreak occurs.

Sera from 945 roe deer were obtained from all over Austria and screened with IFAT for the antibodies against TBE. Twenty-two positive samples, 2.4%, and 17 samples at the borderline titre of 1:16 were identified. The majority of the positive samples, 70.6%, were located in known TBE areas based on human cases. Further research is needed to confirm or reject new endemic foci of TBE transmission

Keywords: TBE Roe deer Risk map

© 2015 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

*Corresponding author. Tel.: +43 1 25077 2211; fax: +43 1 25077 2290.E-mail address: Georg.Duscher@vetmeduni.ac.at (G.G. Duscher).1Tel.: +43 1 25077 2211; fax: +43 1 25077 2290.

http://dx.doi.org/10.1016/j.ttbdis.2015.03.018 1877-959X/© 2015 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Introduction

The *Flavivirus* responsible for causing tick-borne encephalitis (TBE) can be split into three subtypes: the Far-Eastern, the Siberian and the European subtype. The latter subtype can be predominantly found in Central and Northern Europe and Western parts of Russia (Heinz et al., 2013). This virus subtype is mainly transmitted by the vector tick *I. ricinus* (Labuda and Randolph, 1999), and can also be transmitted by raw milk and milk products originating from recently infected goats, sheep or cattle (Holzmann et al., 2009; Labuda et al., 2002).

Annually about 3000 human TBE cases are reported from European countries (Kiffner et al., 2012); estimations worldwide calculate more than 10,000 hospitalized people (WHO, 2011). Nowadays, in 2014, the immunization for TBE – with at least one dose once in their life – of Austrian citizens is about 85% of all inhabitants (GfK Healthcare, 2014). It is assumed that more than 4000 people were prevented from becoming infected with the TBE virus between 2000 and 2011 (Heinz et al., 2013). This high value of vaccinated people is responsible for a low number of TBE cases in Austria with an incidence below 1 per 100,000 inhabitants in 2009 with a vaccination rate in that period of 88% with at least one dose per life (Donoso Mantke et al., 2011; Heinz et al., 2013; Walder et al., 2008). In comparison the neighbouring country of the Czech Republic had a much higher incidence in the same year of 7.8 per 100,000,

reflecting the low immunization rate of 16% of inhabitants. Among the non-vaccinated groups similar incidence rates are observed between Czech Republic and Austria (Heinz et al., 2013). For many reasons such as children's health, tourism and animal health e.g. rare cases in dogs, further efforts in this field is obligatory and mapping of the occurrence of the virus is of major concern. But the areas, so called “foci of transmission”, where the TBE virus (TBEV) circulate, are very restricted due to different requirements on vector and reservoir hosts, which in turn are influenced by habitat parameters such as climate and vegetation (Estrada-Peña and de la Fuente, 2014; Randolph, 2009). Therefore in middle Europe the occurrence of TBEV in ticks is supposed to be clustered in small areas. Consequently integral identification of risk areas is hampered due to the difficulty of discovering all these small foci. Based on data of patients, the current distribution map of TBE in Austria is built and constantly updated (Baxter Healthcare GmbH, Wien). The outcome of this map could be biased due to spatially inhomogeneous immunization rates, and the lack of data from the unsuspicious mild infections of human patients. To overcome these problems, additional strategies were developed. Questing ticks were sampled in various countries and screened for the occurrence of the virus (Dobler et al., 2011; Gäumann et al., 2010; Holbach and Oehme, 2002; Kupča et al., 2010; Rieille et al., 2014; Süss et al., 2004). These investigations delivered important results concerning proven virus distribution and gave the chance to obtain different virus isolates of different regions, ticks and time points. For epidemiological screening the limits in terms of this method are not negligible. First of all the occurrence of the virus is focussed on highly localized areas (Stefanoff et al., 2013), which needs a very small meshed sampling design. This in turn increases the costs of the surveillance, especially in low-risk areas (Süss et al., 2004). In some cases the virus load in questing ticks could be very low and only reach detection limit after replication, which takes place after attachment (Belova et al., 2012). Especially if the samples are pooled, a low virus load might remain undetected, reflecting a wrong picture of the actual virus distribution.

Another study compared the occurrence of human cases with the findings in the ticks and concluded that tick surveillances alone do not deliver reliable data for prediction maps of the TBE virus (Stefanoff et al., 2013).

So, other efforts were conducted to identify indicator species to obtain spatial distribution data. It has been suggested to use wildlife or livestock animals (Stefanoff et al., 2013). The obvious animals therefore are the natural reservoir of the virus, the rodents. Some studies on these showed a sero-conversion of about 2.6% and virus infection 0 of up to 20% (Achazi et al., 2011; Radda et al., 1971; Tonteri et al., 2011). The time-consuming effort needed for the catching and sampling, including a small meshed sample design, is the reason for this study not to choose these species.

In this study the roe deer was chosen to look for the occurrence of TBE for several reasons. The roe deer is distributed all over Austria. It can be found in the lowlands up to a higher level, with an assumed reduction of habitat quality above 1600 m in summer (Reimoser et al., 2009). This species is known to remain in a rather small home range of about 0.16–0.81 km^2 (Jeppesen, 1990; Nosek et al., 1967; Radda et al., 1968b). Roe deer are known as heavy tick carriers (Kiffner et al., 2010; Vor et al., 2010), but symptomatic TBE in roe deer has never been described so far (Nosek et al., 1967; Radda et al., 1968a,b). Sampling can be done on a large scale by instructed hunters. Last but not least several studies on roe deer declared the animals suitable for use as sentinels (Gerth et al., 1995; Kiffner et al., 2012; Nosek et al., 1967; Radda et al., 1968a,b; Skarphédinsson et al., 2005). All these attributes make the roe deer suitable as an indicator species for TBE.
Therefore we designed and conducted a surveillance of Austrian roe deer for the occurrence of TBE all over Austria. The aim of this study was to deliver additional data as a starting point for an integral risk assessment of the virus distribution.

Materials and methods

A total of 2480 sample tubes sent in packages of five tubes were distributed – related to the size of each county – to the hunters with the help of the local hunting organizations. These packages consisted of pre-numbered tubes and form sheets questioning data on sex, estimated age and location of the roe deer. Additionally a prepaid, labelled and addressed envelope was provided in each package to ensure a higher return rate.
945 sera of male and female roe deer, shot between 1st September 2013 and 31st December 2013, were sent to the Institute of Parasitology. These were aliquoted and forwarded to Gernot Walder GmbH for further investigation.

For the production of the IFAs, 25 cm^2 flasks with monolayers of Vero B4 cells (no. ACC-33, DSMZ) were infected with TBE virus strain K306, Westnile virus (WNV) strain Milano 1 or Usutu strain Vienna and incubated at 36 °C by gently shaking the flasks every 10 min. After 1 h, Medium199 (Invitrogen GmbH, Darmstadt, Germany), supplemented with 5% inactivated foetal calf serum (Invitrogen GmbH, Darmstadt, Germany), was added to the cultures. When cytopathic effects were detected, the infected cultures were trypsinized, cells were adhered to IFA slides (GML) for 1 h at 37 °C and then fixed with ice-cold 1:1 acetone-methanol mixture. The percentage of infected cells was adjusted to 50% by adding a certain amount of uninfected cells.
The IFA cut-off titres for TBE were established by analysing 125 sera from roe deer which were shot at least 50 km from the next known focus or residence of a human case of TBE. Among this low-risk collective 20.8% were positive for IgG antibodies at a titre of 1:4, 8% at a titre of

1:8 and 0.8% were positive at 1:16. Thus, according to the criteria of WHO, the cut-off titre was set at 1:16, where at least 98% of negative sera or low-risk sera yield a negative result.

20 µl of diluted sample was applied on the slides and the slides incubated for 40 min at 39 °C, then washed in PBS twice. For detection, 20 µl of FITC-labelled chicken anti-deer IgG antibodies (ACerIG-F, Gallus Immunotech Inc., Ontaria, Canada) were applied on the slide and incubated for 40 min at 39 °C, washed in PBS and covered with Glycerine/PBS 9:1.

Sera were rated positive when the fluorescence signal could be clearly distinguished from background at a 16-fold dilution. Sera were rated as borderline, when they gave a weak fluorescence signal at a 16-fold dilution. Positive and borderline sera were tested by two independent teams comprising of one technically and one microscopically working person each. Sera which were rated positive by both teams were marked as positive and diluted to the endpoint. Sera which were rated negative by at least one team were rated as negative. All other sera (e.g. positive/borderline or borderline/borderline) were rated as questionable. Only the samples tested positive and questionable for TBE, were tested further for Usutu- virus and WNV.

The positions of the roe deer were located on commune level in a map using the geographical information system “arcinfo” (ESRI®arcmap™ 10.0). Results of the IFAT were classified as negative (0), questionable (1) and positive (2). Maps using the inverse distance weighting function of arcinfo were used to draw a map. A catch distance of 10 km between the locations was chosen.
Maps of known TBE cases in humans were inserted in the maps as well to give an integral view (Baxter Healthcare GmbH, Wien). Calculations were performed in Excel® 2002 (Microsoft, Washington) and SPSS v. 20 (SPSS Inc., Chicago, USA). Differences between the groups were analyzed by using the Kruskal–Wallis test.

Results

Of the 945 sera, 22 were positive and 17 were questionable on the borderline of 1:16 titre. Latter questionable sera showed reaction at the cut of level and cannot be counted neither as positive nor as negative samples. All of the 22 positive roe deer sera originated from females (Table 1). The prevalence was 2.4%. Thirteen of the 17 borderline sera were also females. None of the 22 sera positive for TBE were positive for Usutu- or Westnile virus. One questionable serum was tested positive (1:32) for Usutu virus, therefore was left out for further investigations.
Table 1. Age (estimated by the local hunter), sex, positive and questionable (at the borderline titre) roe deer sera. “pos” = TBE antibody positive.

Age	Females/pos/questionable	Males/pos/questionable	Sex unspecified
1	33/2/–	16/–/–	1
2	149/3/2	16/–/–	1
3	211/8/2	16/–/–	2
4	145/5/2	12/–/–	1
5	102/2/2	18/–/3	2
6	60/–/3	8/–/1	–
7	68/–/1	2/–/–	–
8	44/–/–	1/–/–	1
9	6/–/–	–/–/–	–
10	11/–/–	–/–/–	–
11	1/–/–	–/–/–	–
Unspecified	14/2/–	2/–/–	2
Total	**844/22/12**	**91/–/4**	**10**
%	90.3/2.4/1.4	9.7/–/0.4	1.07
Mean age	4.1/3.1/4.4	3.4/–/5.3	3.88
95% CI	[4.0–4.3]/[2.6–3.6]/[3.4–5.5]	[3.1–3.8]/–/[4.4–6.0]	[2.1–5.7]

Fourteen of the 22 positive roe deer sera, 63.6%, are found in supposed human risk areas. Eight of the positive sera were found in areas where no human case has been reported so far. Of the questionable 16 sera at the borderline, 68.8% were found in areas with no human case (Fig. 1). Concerning the age composition there is a significant difference between males and females ($p < 0.01$), but there is no significant difference in terms of positive or questionable sera between males and females.

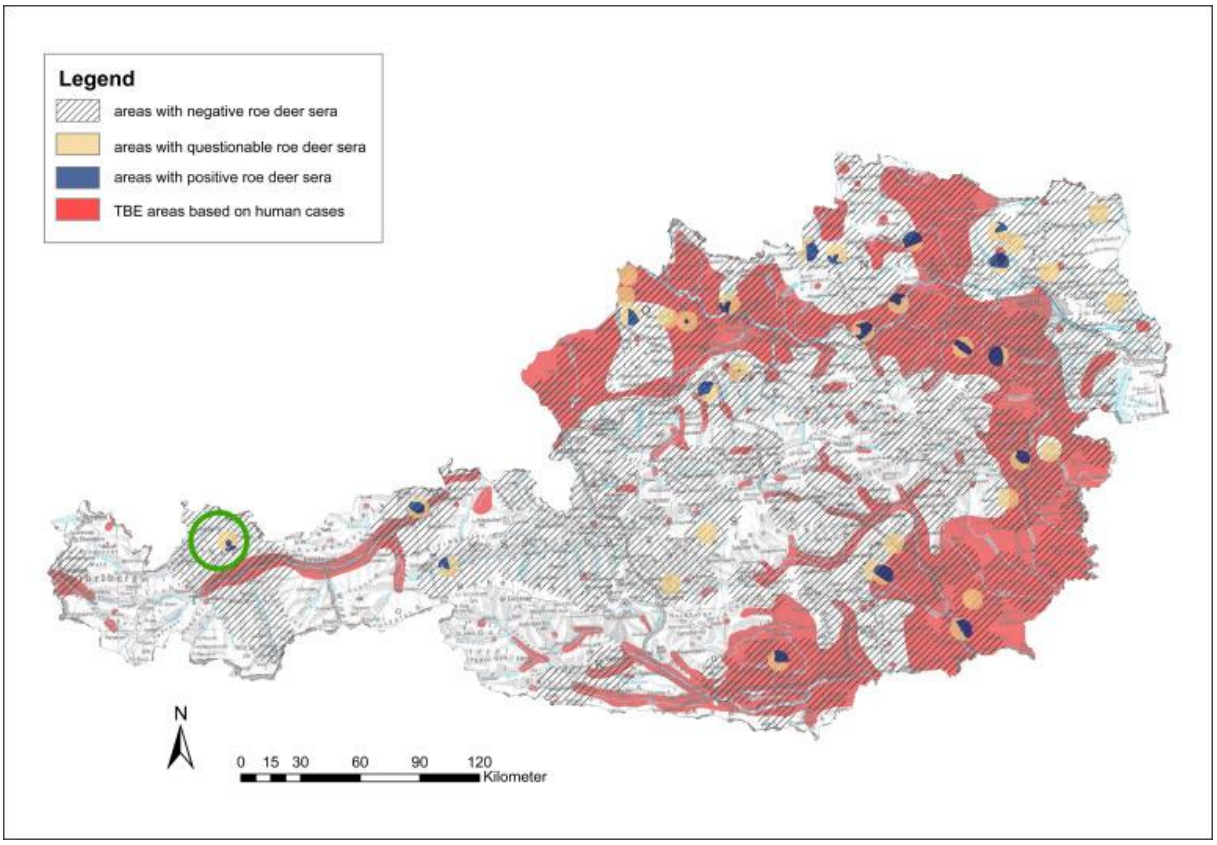

Fig. 1. Map of Austria showing TBE risk areas (Baxter Healthcare GmbH, Wien) and found potential transmission foci based on the roe deer samples. The coloured areas for positive and questionable (at the borderline titre) roe deer sera reflect an estimation based on interpolation of the roe deer sera by inverse distance weighting. The green circle indicates the area of Berwang, where a human seroconverted during the study in a supposed TBE-free region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Discussion

Risk assessment of TBE remains a topic of major concern in terms of public health. Although a good proportion of Austria's citizens are aware of the topic and vaccination rates – rate of people who received at least one dose per life – are incomparably high, these rates seem to have decreased in the recent past from 88% in 2005 to 85% in 2011, to 82% in 2013 and slightly increased to 85% in 2014 again (GfK Healthcare, 2014; Heinz et al., 2013). One important tool for prevention is to provide distribution or risk maps. Nevertheless risk maps for TBE based on human cases can be biased due to the travel behaviour of humans, different vaccination rates and disproportionate exposure risk among vaccinated and non-vaccinated people (Heinz et al., 2013; Kiffner et al., 2012), and also possibly due to a different pathogenicity of different virus isolates in various areas (Dobler et al., 2009; Stefanoff et al., 2013).

In terms of TBE a countrywide surveillance system based on tick sampling seems to be ineffective and time- and cost-consuming, and thus not an ideal method to substitute human risk maps (Stefanoff et al., 2013; Süss et al., 2004). Furthermore it does not reflect the actual risk onto humans, so other surveillance methods such as wildlife or farm animal investigations have been suggested (Stefanoff et al., 2013).

Roe deer represent very good sentinel animals, which can help to substitute risk maps (Gerth et al., 1995; Kiffner et al., 2012; Nosek et al., 1967; Radda et al., 1968a, 1968b). Furthermore it is believed to be one of the driving forces of tick distribution, spreading and sustaining the population (Carpi et al., 2008; Knap and Avšič-Županc, 2013; Labuda and Randolph, 1999; Medlock et al., 2013), therefore might be responsible for he spreading of pathogens as well (Kiffner et al., 2012). In terms of TBE this is discussed controversially: the viraemia in roe deer is supposed to be too low to have an impact on spreading the pathogen itself (Labuda et al., 2002; Nosek et al., 1967). Large ungulates are believed to harbour mainly adult ticks. The vertical transmission of the virus to the progeny seems negligible, presumably not delivering enough infected ticks to implement a new transmission foci (Estrada-Peña and de la Fuente, 2014). Large mammals contribute to the transmission by feeding adult ticks and thus maintaining the tick population (Labuda and Randolph, 1999). Additionally on roe deer all three life stages can be found, feeding (Skarphédinsson et al., 2005) and potential co-feeding between nymphs and females and larvae and nymphs was predicted due to substantial overlapping of attachment sites on the host (Kiffner et al., 2011).

Quite intriguing is the low number of total seroconverted animals compared to other studies, where 15% to up to 50% positive roe deer in an area were detected (Kiffner et al., 2012; Radda et al., 1968b). We tried to achieve sera from many different places to cover more areas. In contrast to the study of Kiffner and colleagues (2012), for example, a much larger area was screened, thus including many areas with no TBEV foci. So, if the “mesh size” of the investigated roe deer samples had been smaller, we would have obtained more animals in and around the positive foci, and consequently the overall prevalence would have increased.

Although there has been a trend of TBE shifting to higher altitudes over the last ten years, natural foci of TBE are usually recorded below 1400 m of altitude, yielding a relatively small percentage of potentially affected areas in the alpine parts of the country (Walder et al., 2008). Additionally the sex might have an impact on the result. Interestingly in one study females were less positive concerning antibodies than male roe deer, for which the authors cannot give an explanation (Gerth et al., 1995). Although there are no differences in the home range of the sexes, this might be reflected by the habit of movement of the animals. Males have bigger activity patterns in springtime, probably as a result of territorial behaviour (Jeppesen, 1990). So, males might have an increased likelihood of achieving a positive focus at a time of the year

with ongoing tick activity. In our data set we could not confirm a higher risk for males of becoming infected.
A third explanation for the low number of positive roe deer sera could lie in the material itself. The blood of the roe deer often had a haemolytic appearance due to the logistical challenges, so antibodies might have denatured as well. Although this cannot be ruled out, the antibodies are known to be very stable and we do not expect this to have a large impact.

The majority of positive roe deer sera (63.6%) were from the areas of known or assumed TBE risk based on human cases. This can be seen not only as confirmation of the already existing map, but also as additional information giving an integral view on possible risk in the already known areas. Due to the fact that roe deer do not migrate like humans do in the form of tourism and the like, positive foci could be identified more accurately. With the help of this information, attempts can be undertaken to localize transmission foci.

Much more important are the areas which were identified being potential risk areas based on the roe deer sera data, but have not shown any human cases in the past. These areas might have been neglected in terms of human infections, possibly due to fewer human visits based on lower "attractiveness" for humans (Estrada-Peña and de la Fuente, 2014) such as uninviting landscape, dense vegetation etc. Humans might not have reached these foci until now, whereas roe deer are living there, "sampling" positive ticks. If these areas are not attractive for human use, the actual risk coming from these areas can be discussed. Due to the increasing popularity of outdoor activities of people expanding to new and undiscovered places, any of these new potential foci should be considered in terms of potential TBE refuges. Similar to the other foci, these localized areas help to get an integral view on possible infection sites.
Notable is the fact that there is at least one potential focus based on the roe deer data in Berwang (marked with a green circle in Fig. 1), which overlaps geographical with a serologically confirmed human case, who has probably been acquired about 10–15 km away in the neighbouring community of Ehrwald during the course of this study (Walder, unpublished). This area had hitherto not been known to host TBE.
But caution has to be exercised with these data. Similarly to human case data, the roe deer data can be biased. Normally the movement of roe deer is supposed to be restricted to a certain area and during a study of tagged roe deer fawns, 64.7% stayed within 500 m of their birthplace. About 2.1% moved more than 20 km, but some individuals might migrate up to 64 km (Reimoser et al., 1999). Especially young deer migrate some distance, until they find their habitat. But TBE antibodies are believed to persist a lifetime (Stanek and Hofmann, 1994). So it cannot be excluded that young roe deer become infected in a certain area, then moved to another and settled there. This might draw a wrong picture of non-existing transmission foci. Therefore the location of one positive roe deer serum is not equal to one positive infection

focus. Even more, there might be possibility concerning cross-reactions to other flavivirus such as Usutu- and Westnile virus. In the cases of the positive TBE sera found in this study, we could exclude this for the aforementioned viruses. One questionable serum was positive for Usutu, therefore the borderline TBE titre might reflect a wrong positive result for TBE. Nevertheless the others did not reveal a positive result neither for Usutu- nor for Westnile virus. So, even if we exclude the serum tested positive for Usutu from all questionable sera in unknown risk areas, there is still a high amount of 68.8% of these sera remaining. This might be a hint for a low infection pressure in these cases. It is generally assumed that roe deer become re-infected every now and then if situated near a transmission foci, consequently the antibody titre remains at a high level (Nosek et al., 1967). The questionable titre might be caused by a lack of re-infections due to an inability to reach positive ticks as a consequence of migration to a TBE-free region. Yet there are no long term data on antibody persistence in roe deer after TBE infection, therefore further investigations on other roe deer, rodents and ticks have to be made to confirm a positive site. Additionally the data reflect a snapshot and do not claim to deliver all positive foci of transmission in Austria, meaning that areas with confirmed negative roe deer do not necessary exclude the occurrence of any TBE foci in that area.

Concerning the age of the roe deer, it is assumed that it does not have a big impact on the results, because if situated near to a focus, the animals become infected quite early. If there is no infection foci, the roe deer will not be exposed during their entire life (Gerth et al., 1995). This is in concordance with this data. The animals with a positive titre have a lower mean age than the whole group, indicating a very early infection time point.

In conclusion roe deer represent a very good indicator species due to the easiness of obtaining samples, the overall distribution of the roe deer and the almost restricted home range of the animals. Especially in areas of high vaccination rates of inhabitants, such as in Austria, this method should be considered as additional data on the distribution of the virus. Only a combination of all available direct and indirect data e.g. from humans, ticks, rodents and wild ungulates is able to give an integral view on the actual distribution of the virus.

Acknowledgements

We thank Mag. Liz Avedano and Roman Peschke for assistance in the lab and with analysis. Additionally we thank Baxter Healthcare GmbH, Wien for substitution and the hunting organizations of Austria for their help.

The work was done under the frame of EurNegVec COST Action TD1303.

References

Achazi et al., 2011
K. Achazi, D. Růžek, O. Donoso-Mantke, M. Schlegel, H.S. Ali, M. Wenk, J. Schmidt-Chanasit, L. Ohlmeyer, F. Rühe, T. Vor, C. Kiffner, R. Kallies, R.G. Ulrich, M. Niedrig. Rodents as sentinels for the prevalence of tick-borne encephalitis virus. Vector Borne Zoonotic Dis., 11 (2011), pp. 641-647, 10.1089/vbz.2010.0236. CrossRefView Record in Scopus

Belova et al., 2012
O.A. Belova, L.A. Burenkova, G.G. Karganova. Different tick-borne encephalitis virus (TBEV) prevalences in unfed versus partially engorged ixodid ticks – evidence of virus replication and changes in tick behavior. Ticks Tick. Borne. Dis., 3 (2012), pp. 240-246, 10.1016/j.ttbdis.2012.05.005. ArticleDownload PDFView Record in Scopus

Carpi et al., 2008
G. Carpi, F. Cagnacci, M. Neteler, A. Rizzoli. Tick infestation on roe deer in relation to geographic and remotely sensed climatic variables in a tick-borne encephalitis endemic area. Epidemiol. Infect., 136 (2008), pp. 1416-1424, 10.1017/S0950268807000039. View Record in Scopus

Dobler et al., 2011
G. Dobler, F.T. Hufert, M. Pfeffer, S. Essbauer. Tick-borne encephalitis: from microfocus to human disease. Prog. Parasitol. Parasitol. Res. Monogr. (2011), p. 2. View Record in Scopus

Dobler et al., 2009
G. Dobler, A. Kupca, A. Zöller, R. Brey, S. Essbauer, M. Pfeffer, P. de Mendonca. Temporal and spatial dynamics of a highly active tick-borne encephalitis (TBE) focus in south-eastern Germany: isolation and molecular characterization of an unusual TBE virus strain Gerhard Dobler. X International Jena Symposium on Tick-Borne Diseases (2009), p. 66. View Record in Scopus

Donoso Mantke et al., 2011
O. Donoso Mantke, C. Escadafal, M. Niedrig, M. Pfeffer, Working Group for Tick-Borne Encephalitis Virus C. Tick-borne encephalitis in Europe, 2007 to 2009. Euro Surveill. (2011), p. 16

Estrada-Peña and de la Fuente, 2014
A. Estrada-Peña, J. de la Fuente. The ecology of ticks and epidemiology of tick-borne viral diseases. Antiviral Res., 108C (2014), pp. 104-128, 10.1016/j.antiviral.2014.05.016. ArticleDownload PDFView Record in Scopus

Gäumann et al., 2010
R. Gäumann, K. Mühlemann, M. Strasser, C.M. Beuret. High-throughput procedure for tick surveys of tick-borne encephalitis virus and its application in a national surveillance study in Switzerland. Appl. Environ. Microbiol., 76 (2010), pp. 4241-4249, 10.1128/AEM.00391-10. CrossRefView Record in Scopus

Gerth et al., 1995
H.J. Gerth, D. Grimshandl, B. Stage, G. Doller, C. Kunz. Roe deer as sentinels for endemicity of tick-borne encephalitis virus. Epidemiol. Infect., 115 (1995), pp. 355-365. CrossRefView Record in Scopus

GfK Healthcare, 2014
GfK Healthcare. Impfstatus 2014 (2014)

Heinz et al., 2013

F.X. Heinz, K. Stiasny, H. Holzmann, M. Grgic-Vitek, B. Kriz, A. Essl, M. Kundi. Vaccination and tick-borne encephalitis, central Europe. Emerg. Infect. Dis., 19 (2013), pp. 69-76, 10.3201/eid1901.120458. CrossRefView Record in Scopus

Holbach and Oehme, 2002
M. Holbach, R. Oehme. FSME und Lyme-Borreliose. Erregerverbreitung in Zecken und Erkrankungsrisiken in einem FSME-Risikogebiet. Fortschr. Med. Orig., 120 (2002), pp. 113-118. View Record in Scopus

Holzmann et al., 2009
H. Holzmann, S.W. Aberle, K. Stiasny, P. Werner, A. Mischak, B. Zainer, M. Netzer, S. Koppi, E. Bechter, F.X. Heinz. Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. Emerg. Infect. Dis., 15 (2009), pp. 1671-1673, 10.3201/eid1510.090743. CrossRefView Record in Scopus

Jeppesen, 1990
J. Jeppesen. Home range and movements of free-ranging roe deer (*Capreolus capreolus*) at Kalø. Danish Rev. Game Biol., 14 (1990), pp. 2-14

Kiffner et al., 2010
C. Kiffner, C. Lödige, M. Alings, T. Vor, F. Rühe. Abundance estimation of Ixodes ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). Exp. Appl. Acarol., 52 (2010), pp. 73-84, 10.1007/s10493-010-9341-4. CrossRefView Record in Scopus

Kiffner et al., 2011
C. Kiffner, C. Lödige, M. Alings, T. Vor, F. Rühe. Attachment site selection of ticks on roe deer, *Capreolus capreolus*. Exp. Appl. Acarol., 53 (2011), pp. 79-94, 10.1007/s10493-010-9378-4. CrossRefView Record in Scopus

Kiffner et al., 2012
C. Kiffner, T. Vor, P. Hagedorn, M. Niedrig, F. Rühe. Determinants of tick-borne encephalitis virus antibody presence in roe deer (*Capreolus capreolus*) sera. Med. Vet. Entomol., 26 (2012), pp. 18-25, 10.1111/j.1365-2915.2011.00961.x. CrossRefView Record in Scopus

Knap and Avšič-Županc, 2013
N. Knap, T. Avšič-Županc. Correlation of TBE incidence with red deer and roe deer abundance in Slovenia. PLoS One, 8 (2013), p. e66380, 10.1371/journal.pone.0066380. CrossRef

Kupča et al., 2010
A.M. Kupča, S. Essbauer, G. Zoeller, P.G. de Mendonça, R. Brey, M. Rinder, K. Pfister, M. Spiegel, B. Doerrbecker, M. Pfeffer, G. Dobler. Isolation and molecular characterization of a tick-borne encephalitis virus strain from a new tick-borne encephalitis focus with severe cases in Bavaria, Germany. Ticks Tick. Borne. Dis., 1 (2010), pp. 44-51, 10.1016/j.ttbdis.2009.11.002. ArticleDownload PDFView Record in Scopus

Labuda et al., 2002
M. Labuda, E. Elečková, M. Ličková, A. Sabó. Tick-borne encephalitis virus foci in Slovakia. Int. J. Med. Microbiol., 291 (2002), pp. 43-47, 10.1016/S1438-4221(02)80008-X. ArticleDownload PDFView Record in Scopus

Labuda and Randolph, 1999
M. Labuda, S.E. Randolph. Survival strategy of tick-borne encephalitis virus: cellular basis and environmental determinants. Zentralblatt für Bakteriol., 289 (1999), pp. 513-524, 10.1016/S0934-8840(99)80005-X. ArticleDownload PDFView Record in Scopus

Medlock et al., 2013

J.M. Medlock, K.M. Hansford, A. Bormane, M. Derdakova, A. Estrada-Peña, J.-C. George, I. Golovljova, T.G.T. Jaenson, J.-K. Jensen, P.M. Jensen, M. Kazimirova, J.A. Oteo, A. Papa, K. Pfister, O. Plantard, S.E. Randolph, A. Rizzoli, M.M. Santos-Silva, H. Sprong, L. Vial, G. Hendrickx, H. Zeller, W. Van Bortel. Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. Parasit. Vectors, 6 (2013), p. 1, 10.1186/1756-3305-6-1. CrossRef

Nosek et al., 1967
J. Nosek, O. Kozuch, E. Ernek, M. Lichard. Übertragung des Zeckenencephalitis-Virus (TBE) duch die Weibchen von *Ixodes ricinus* und Nymphen von *Haemaphysalis inermis* auf die Rehkitzen (*Capreolus capreolus*). Zentralbl. Bakteriol. Orig., 203 (1967), pp. 162-166.
View Record in Scopus

Radda et al., 1971
A. Radda, H. Hofmann, O. Kozuch, C. Kunz, J. Nosek, K. Zukrigl. Ein Naturherd des Frühsommer-Meningoenzephalitis-Virus bei St. Florian. Anzeiger für Schädlingskd. und Pflanzenschutz, 44 (1971), p. 96, 10.1007/BF02040652. CrossRefView Record in Scopus

Radda et al., 1968a
A. Radda, H. Hofmann, C. Kunz. Experimentelle Infektion einiger heimischer Saugerarten mit dem Frühsommer-Meningo-Enzephalitis (FSME)-Virus. Zentralbl. Bakteriol. Orig., 208 (1968), pp. 100-104. View Record in Scopus

Radda et al., 1968b
A. Radda, C. Kunz, H. Hofmann. Nachweis von Antikörpern in Wildseren zur Erfassung von Herden des Virus der Frühsommer-Meningo-Enzephalitis FSME) in Niederösterreich. Zentralbl. Bakteriol. Orig., 208 (1968), pp. 88-93. View Record in Scopus

Randolph, 2009
S.E. Randolph. Tick-borne disease systems emerge from the shadows: the beauty lies in molecular detail, the message in epidemiology. Parasitology, 136 (2009), pp. 1403-1413, 10.1017/S0031182009005782. CrossRefView Record in Scopus

Reimoser et al., 1999
F. Reimoser, J. Zandl, D. Winkler. Rehkitzmarkierung. Weidwerk, 10 (1999), pp. 26-28. View Record in Scopus

Reimoser et al., 2009
S. Reimoser, E. Partl, F. Reimoser, S. Vospernik. Roe-deer habitat suitability and predisposition of forest to browsing damage in its dependence on forest growth—Model sensitivity in an alpine forest region. Ecol. Modell., 220 (2009), pp. 2231-2243, 10.1016/j.ecolmodel.2009.05.022. ArticleDownload PDFView Record in Scopus

Rieille et al., 2014
N. Rieille, S. Bressanelli, C.C.M. Freire, S. Arcioni, L. Gern, O. Péter, M.J. Voordouw. Prevalence and phylogenetic analysis of tick-borne encephalitis virus (TBEV) in field-collected ticks (*Ixodes ricinus*) in southern Switzerland. Parasit. Vectors, 7 (2014), p. 443, 10.1186/1756-3305-7-443. CrossRef

Skarphédinsson et al., 2005
S. Skarphédinsson, P.M. Jensen, K. Kristiansen. Survey of tickborne infections in Denmark. Emerg. Infect. Dis., 11 (2005), pp. 1055-1061. CrossRefView Record in Scopus

Stanek and Hofmann, 1994
G. Stanek, H. Hofmann. Krank durch Zecken. Verlag Wilhelm Maudrich, Wien, München, Bern (1994)

Stefanoff et al., 2013
P. Stefanoff, M. Pfeffer, W. Hellenbrand, J. Rogalska, F. Rühe, A. Makówka, J. Michalik, B. Wodecka, A. Rymaszewska, D. Kiewra, A. Baumann-Popczyk, G. Dobler. Virus detection in questing ticks is not a sensitive indicator for risk assessment of tick-borne encephalitis in humans. Zoonoses Public Health, 60 (2013), pp. 215-226, 10.1111/j.1863-2378.2012.01517.x. CrossRefView Record in Scopus

Süss et al., 2004
J. Süss, C. Schrader, U. Falk, N. Wohanka. Tick-borne encephalitis (TBE) in Germany – epidemiological data, development of risk areas and virus prevalence in field-collected ticks and in ticks removed from humans. International Journal of Medical Microbiology (Suppl.) (2004), pp. 69-79. ArticleDownload PDFView Record in Scopus

Tonteri et al., 2011
E. Tonteri, A.E. Jääskeläinen, T. Tikkakoski, L. Voutilainen, J. Niemimaa, H. Henttonen, A. Vaheri, O. Vapalahti. Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008–2009. Emerg. Infect. Dis., 17 (2011), pp. 72-75, 10.3201/eid1701.100051. CrossRefView Record in Scopus

Vor et al., 2010
T. Vor, C. Kiffner, P. Hagedorn, M. Niedrig, F. Rühe. Tick burden on European roe deer (*Capreolus capreolus*). Exp. Appl. Acarol., 51 (2010), pp. 405-417, 10.1007/s10493-010-9337-0. CrossRefView Record in Scopus

Walder et al., 2008
G. Walder, B. Falkensammer, F.X. Heinz, H. Holzmann, M.P. Dierich, R. Würzner. Tick-borne encephalitis in the Tyrol (Austria): changes in incidence and endemicity 2000–2006. Int. J. Med. Microbiol., 298 (2008), pp. 88-93, 10.1016/j.ijmm.2008.05.002. ArticleDownload PDFView Record in Scopus

WHO, 2011
WHO. Vaccines against tick-borne encephalitis: WHO position paper. Wkly. Epidemiol. Rec. Relev. épidémiologique Hebd. 2011 (2011), pp. 241-256

3. Kapitel - Serological and molecular screening for tick-borne zoonotic pathogen *Babesia* spp. in hunters in Tyrol, Austria

(Wetscher et al., 2020)

Autoren: Monika Wetscher. Klaus Hackländer, Viktoria Faber, Anna Maria Dieplinger, Arno Michael Lechner, Julia Walochnik, Georg Gerhard Duscher

Journal: Vector Borne Diseases

Kurzfassung

In der vorliegenden Studie wurden Blutproben von 813 aktiven gesunden JägerInnen in allen neun Bezirken Tirols gewonnen. Sie wurden serologisch mittels PCR auf anti-*Babesia*-Antikörper und *Babesia* spp. untersucht. Von diesen 813 JägerInnen erinnerten sich 550 (68%) an einen Zeckenstich. 755 (93%) jagen regelmäßig Schalenwild. Anti-*Babesia*-Antikörper wurden mittels IFAT in einem einzigen Serum (0,12%) der 813 Proben mit einem Titer von 1:64 nachgewiesen. In 28 Seren (3,4%) wurde ein Titer von 1:16 bestimmt. Alle PCR Proben waren negativ auf *Babesia* spp.

Die Resultate bestätigen das Vorhandensein von anti-*Babesia*-Antikörpern bei gesunden Personen. Die Abwesenheit von DNA spricht für eine hohe Selbstlimitierung bei diesen asymptomatischen und immunkompetenten Individuen.

Wichtigste Ergebnisse der Untersuchung

Ein Kontakt zu den zoonotischen Erregern konnte in dieser Studie klar gezeigt werden. Von den 813 mittels IFAT getesteten Seren reagierten 28 (3,4%; 95% CI: 2,2-4,7%) auf *Babesia* spp., davon 27 mit einem Titer von 1:16 und 1 Probe mit einem Titer von 1:64. Von den 28 serologisch reagierenden JägerInnen erinnerten sich 20 (71,4%) an einen Zeckenstich. 27 (96,4%) JägerInnen jagen regelmäßig Schalenwild.

Alle reagierenden Proben wurden auch auf das Vorhandensein von Babesia-DNA getestet. Sie alle lieferten in der PCR negative Ergebnisse. Auf Bezirksebene zeigten Kitzbühel (6%, 95% CI: 1-11%) und Landeck mit (7%, 95% CI: 2-12%) in der Seroprävalenz die höchsten Werte. Geringe Seroprävalenz wurde hingegen in der Region Innsbruck und Innsbruck-Land festgestellt.

Hochrisikogebiete zu definieren ist jedoch aufgrund der allgemein niedrigen Prävalenz schwierig.

Erkenntnis zur Fragestellung

Unsere primäre Frage war, ob wenig bekannte Pathogene, die durch Vektoren übertragen werden, in Österreich existent sind. Diese Frage konnten wir in dieser Arbeit umsetzen und beantworten.

In unserem Fokus stand die Untersuchung von *Babesien* spp.

Informationen über diese Erreger sind insofern sehr wichtig und bedeutungsvoll, da sich die Therapie dieser von ihnen verursachten Erkrankung von der Borreliose sehr unterscheidet. Diese Studie soll vor allem praktizierenden Ärzten helfen auch an diese Erreger zu denken, sie in ihre Differentialdiagnose miteinzubeziehen und dadurch die richtige Behandlung einzuleiten.

Original Manuskript

Wurde am 14 Jänner 2020 akzeptiert, Manuskript Nummer: jvbd_145_19

Nachfolgend das Mail von Vector Borne Diseases und das eingereichte noch nicht veröffentlichte Manuskript

journalonweb.com/jvbd/showmail.asp?id=C087A794872640l6BEE2D28F423221DCCAEAE54F2BCFC18BE48FCBA352A45F49

EMail

Showing mail for : m.wetscher@gmx.at

Date	Tuesday, January 14, 2020 11:26:55 AM GMT
Subject	[jvbd]:Decision on your article:jvbd_145_19
Message	If you cannot see this page properly, please click here.

Dear Miss. Wetscher,

NOTE: This e-mail is sent to you as one of the contributing authors. If you are not corresponding author, please co-ordinate with the author designated by your group as the corresponding author for this manuscript

Status of the manuscript titled 'Serological and molecular screening for tick-borne zoonotic pathogen Babesia spp. in hunters in Tyrol, Austria' submitted by Dr. Georg Duscher has been changed and a copy of the mail is as;

Dear Dr. Duscher,

The Editorial Board of Journal of Vector Borne Diseases is pleased to inform you that your manuscript entitled Serological and molecular screening for tick-borne zoonotic pathogen Babesia spp. in hunters in Tyrol, Austria, with manuscript number jvbd_145_19, is acceptable for publication in the Journal.

We will be sending you the page proofs through the manuscript management site before publication of the manuscript or through e-mail. If you have not sent the copyright form signed by all the contributors and the images, if any, till now, you are requested to do so at the earliest. Copyright form can be emailed to editor.jvbd@mrcindia.org or jvbd.nimr@yahoo.co.in. Please note that the journal reserves the rights to make changes in the language, grammar, presentation, etc. to suit the journal's requirements.

We thank you for submitting your valuable research work to Journal of Vector Borne Diseases.
With warm personal regards,

Yours sincerely,

The Editorial Team

Journal of Vector Borne Diseases

Remarks:

Message sent on Tuesday, January 14, 2020

Please add **editor@jvbd.org** as a contact in your E-mail client to ensure that this mail is not considered as a junk mail.

---- END OF MESSAGE ----

Editor
Journal of Vector Borne Diseases
ICMR錘ational Institute of Malaria Research
Sector 8, Dwarka,
New Delhi-110 077
India

Website: http://www.jvbd.org/
E-mail: editor.jvbd@mrcindia.org
Tel: 91-11-25307163
91-11-25365774

Title of the article: Serological and molecular screening for tick-borne zoonotic pathogen *Babesia* spp. in hunters in Tyrol, Austria

Aims: The aim of the study is the assessment of the tick-borne pathogen *Babesia* spp. in active hunters in Tyrol. The epidemiological risk of this zoonotic pathogen was analysed using a serological and molecular screening and may, therefore, support physicians and public authorities in health risk management among this highly exposed group.

Settings and Design: In the present study a cross-sectional study was carried out from March to April 2015 and 2016, comprising all nine districts of Tyrol.

Methods and Material:Blood samples of 813 active hunters were collected and tested for anti-*Babesia* antibodies (*B. divergens* and *B. venatorum*) by serology and for *Babesia* spp. by PCR.
Results: Healthy participants (n=813) between 18 and 86 years of age were tested in this study (median age 52.5 years). Out of these, 755 (93%) regularly hunt game and 550 (68%) recalled having had a tick bite. Of the 813 sera tested by immune fluorescence antibody testing (IFAT), 28 (3.4%; 95% confidence interval (CI): 2.2–4.7%) reacted. Of those only one individual had a titre level of 1:64, which may be interpreted as positive, and 27 (3.32%) had a titre level of 1:16, which is considered negative. By PCR, all samples remained negative for *Babesia* spp. DNA. At the district level, seroprevalence IFAT 1:16 shows the highest values in Kitzbühel with (5.1%, 95% CI: 1-11%) and Landeck with (7.3%, 95% CI: 2-12%).
Conclusions:
To our knowledge this is the first *in vivo* serological screening in Austria conducted during early spring when tick activity is still low. We could show that anti-*Babesia* antibodies were detected in a single healthy tested person. Since these pathogens occur very rarely, our study aimed to focus clinical attention on these rare zoonotic pathogens to facilitate early disease recognition.

Key-words: *Babesia* spp.; serological and molecular screening; hunters; Tyrol; Austria

Key Messages: Since these pathogens occur very rarely, our study aim is intended to help clinicians to become aware about these zoonotic pathogens in order to detect possible cases of illness early enough, and should be considered in the differential diagnosis of febrile illness occurring after exposure to ticks, in particular in immunocompromised patients.

Introduction:

Human babesiosis is a zoonosis with increasing incidence worldwide and is primarily caused by the bite of Ixodid ticks[1]. The tick-borne piroplasmids of the genus *Babesia* spp. (phylum Apicomlexa) are hemoprotozoa[2-4], and several species, including *B. divergens, B. venatorum,* and *B microti*, may cause human babesiosis[5]. Infections due to *Babesia* spp. are particularly dangerous for immunocompromised and functionally or anatomically asplenic individuals, in whom they can be fatal[6]. Blood products can also be contaminated with *Babesia* spp., resulting in further infections[7-8]. *B. divergens* and *B. venatorum* can lead to moderate and severe babesiosis. Patients' symptoms include fever, anemia, hemoglobinemia, respiratory distress syndrome, pulmonary edema, disseminated intravascular coagulation, congestive heart failure, hemoglobinuria, renal failure, coma or splenic rupture but may also show a prolonged relapsing course of illness. However, it has also been shown that *B. venatorum* can also be asymptomatic[22, 24, 26-27]. Different antibody levels for *B. divergens* and *B. venatorum* in the literature are difficult to interpret, and cross-reactions are possible[1, 26]. For treatment, antibiotics such as atovaquone plus azithromycin or clindamycin plus quinine are applied[26]. Although the two most important treatment regimens generally still appear effective, there may be problems with the response speed to therapy[24]; therefore, monitoring of the course of therapy is necessary[27].

Ixodes ricinus (*I. ricinus*), the most common and the most widespread tick species in Austria, is not only the main vector for the tick-borne-encephalitis virus and *Borrelia* spp. but also the main vector for anthropozoonotic *Babesia* spp.[9-11] Coinfections are widespread in *I. ricinus* and may also affect humans, in particular, when there is frequent exposure to tick bites[12-16]. Likewise, the evidence of *Babesia* spp. in wild ungulates is reported as a possible source of infection[28-30].

In contrast, borreliosis has an estimated number of approximately 50,000 human cases in Austria each year[9], so practitioners are usually aware of this bacterial infection. However, knowledge about the epidemiology of infections caused by *Babesia* spp. in Austria is scarce, and to the best of our knowledge, there was only one seroepidemiological study that focused on the risk of transfusion-related babesiosis[15].

The objective of the present study was to conduct a serological and molecular screening for the tick-borne pathogen *Babesia* spp. in active hunters in Tyrol, Austria, in order to contribute to the health risk management of tick-borne diseases, provide information to physicians about this zoonotic pathogen, and raise awareness as to potential therapeutic considerations in addition to other bacterial transmitted tick-borne pathogens.

Subjects and Methods:

Design and study site: A cross-sectional descriptive study was carried out from March to April 2015 and 2016 in all nine districts of Tyrol, Austria. Tyrol is bordered by the provinces of Salzburg to the east, Vorarlberg to the west, and Carinthia to the south. The neighbouring countries of Tyrol are Germany (to the north), Italy (to the south), and Switzerland (to the west). (Fig. 1). Each participant completed a questionnaire that included the patient's age, exposures to tick over the last 5 years, and activities of hunting of wild ungulates (comprising red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), alpine chamois (*Rupicapra rupicapra*) and alpine ibex (*Capra ibex*)).

Selection of human subjects: The selected participants were 813 active hunters (i.e., 5%, 736 males and 77 females). After written informed consent, blood samples were collected during March to April 2015 and 2016.

Sampling and laboratory analysis: The venous blood samples were collected by medical doctors using Vacuette®–Multiple Use Drawing Needle (Greiner Bio-One GmbH. 4550 Kremsmünster, Austria) and Vacuette®–8 ml Z Serum Sep Clot Activator (Greiner Bio-One GmbH. 4,550 Kremsmünster, Austria) and Vacuette®–3ml K3EDTA tubes (Greiner Bio-One GmbH. 4550 Kremsmünster, Austria). The samples were stored at +4°C before further processing.

Serum tubes and questionnaires were numbered and checked twice by independent persons (medical doctors and a principal investigator) for their reliability.

Serological diagnosis: All sera were tested for anti-*Babesia* antibodies by an in-house immunofluorescent antibody test (IFAT) using *B. divergens* as an antigen.[16-17] The bovine-derived *B. divergens* whole cell antigen was fixed onto glass slides. Patient sera were diluted in microtitre plates (1:16, 1:64, 1:256, 1:1024 and 1:4096) in phosphate buffered saline (PBS), 20 µl of each dilution were inoculated onto the reaction sites of the slides and incubated in a moist chamber at room temperature for 40 minutes. Slides were washed twice for seven minutes in PBS, rinsed in distilled water (dH2O) and air-dried. A commercial fluorescein isothiocyanate-conjugated (FITC) goat anti-human antibody to total immunoglobulin (Fluoline H conjugates Biomerieux Vienna, Austria) was used. The conjugate antibody (20 µl) was added at a dilution of 1:400 in PBS and supplemented with 0.5% Evans blue (BioMerieux Vienna, Austria). The slides were again incubated for 40 min in a moist chamber at room temperature, washed twice with PBS (seven minutes), rinsed with dH2O and covered with phosphate-buffered glycerine and a cover slide. Titres were evaluated by fluorescence microscopy (Eclipse E800, Nikon, Vienna, Austria).

A serum sample from a patient with proven babesiosis was used as a positive control; in this case of *B. venatorum* babesiosis confirmed by IFAT, microscopy, PCR and DNA sequencing, PBS was used as a negative control. All sera were tested in the dilutions (in PBS) of 1:16 and

1:64. Therefore, 20 µl of each dilution was inoculated onto the coated slides, incubated for 40 min in a moist chamber at room temperature, washed twice in PBS for seven minutes, rinsed in dH2O and covered with phosphate-buffered glycerine and a coverslip. Subsequently, the 20 µl conjugate was added at a dilution of 1:400 in PBS and supplemented with 0.5%. Evans blue (BioMerieux, Vienna Austria). The binding of antibodies was evaluated by UV microscopy at 400x magnification. All reacted sera (≥1:16) were also tested in the dilutions 1:256, 1:1024 and 1:4096.

Molecular diagnosis: All serologically reacted individuals were also tested for the presence of *Babesia* DNA by PCR using 400 µl EDTA blood samples. In brief, total DNA was isolated from EDTA blood samples using the peqGOLD Blood DNA Mini Kit (Peqlab, Erlangen, Germany) and following the manufacturer's instructions. Isolated DNA out of 400 µl of EDTA whole blood was eluted in 100 µl buffer. A PCR was performed, amplifying an approximately 500 bp fragment of the ribosomal DNA, including the internal transcribed spacer region 1 (ITS1), length depending on species, and allowing species identification after DNA sequencing[18]. One µl, three µl and six µl of the extracted DNA were used for each sample. Double-distilled DNA-free water (Sigma-Aldrich GmbH, Vienna, Austria) was used as a negative control. The amplicons were visualised in a 2% agarose gel by staining with GelRed™ (BioTrend, Cologne, Germany).

Statistical analysis: All analytical experiments were performed in triplicate and repeated three times with similar results. Positive and negative controls were used at all times (see Materials and Methods). A joint datasheet was generated containing age, tick exposures, hunting wild ungulates, as well as serological and PCR test results of hunters. Mean, median and standard deviation were calculated using Microsoft Excel version 2013. The calculation of seroprevalence in the districts was carried out using SPSS package v. 24 (SPSS Inc., Chicago, IL,USA).

Results:

A total of 813 healthy participants were tested in this study (median age 52.5 years). Of these, 550 (68%) recalled having had, at least, one tick bite during the last five years and 755 (93%) of the participants regularly hunted wild ungulates.

Of the 813 sera tested by IFAT, 28 (3.4%; 95% CI: 2.2–4.7%) reacted for *Babesia* spp., of which 27 reacted with a titre of 1:16, and 1 sample reacted with a titre of 1:64. Of the 28 serologically reactive hunters, 20 (71.4%) recalled having had a tick bite and 27 (96.4%) regularly hunt game.

All reactive specimens were further tested for the presence of *Babesia* DNA delivering negative results by PCR. At the district level, the seroprevalence IFAT 1:16 showed the highest values in Kitzbühel with (5.1%, 95% CI: 1-11%) and Landeck with (7.3%, 95% CI: 2-12%). In contrast,

low seroprevalence was seen in the region of Innsbruck and Innsbruck-Land. However, due to the low general prevalence defining high risk regions based in the presented evidence is difficult (see Table 1 and Figure 1)

Discussion:

The awareness and reports of *Babesia* spp. in animals and humans surged in the recent past. The impact of zoonotic *Babesia* spp. onto humans, particularly, remains unclear, and knowledge about spatial and temporal occurrence is scarce[1, 28]. In terms of blood donations, these pathogens pose a potential risk;[15] therefore, screening of apparently healthy people with known outdoor activity represents an indispensable dataset. Even more, 550 (68%) of the 813 (100%) examined hunters, reported having had tick bites in recent years.

In Austria the competent vector of human-relevant Babesia *Ixodes ricinus* is widespread[9] and known to harbour *Babesia* spp. in some areas with prevalences up to 50%[11]. It is quite intriguing that so far only one confirmed autochthonous human babesiosis was diagnosed[22], especially when it is taken into consideration that tick bites are widespread in the Austrian population as shown by the estimated number of 50,000 Lyme cases, another tick-borne pathogen transmitted by *I. ricinus*, per year.[9]

In our study one individual reacted highly positive since a titer of 1:64 found in the first reported case of babesiosis in Austria occurred in 2000 in a splenectomised individual; the causative agent is referred to as EU1 *aka* and later described as the new species *B. venatorum*. This patient had an antibody titre of 1:64 in an IFAT using *B. divergens* as an antigen[22] and this case was also confirmed by using PCR. With the detection of *Babesia* spp. with a titre level of 1:64 in our study, this test procedure has already been used in other studies[15, 22]. Nevertheless, it has to be mentioned that according to WHO guidelines, a titre range of 1:128 for *B. divergens* is to be regarded as positive[15]. Furthermore, 27 samples showed a titre of 1:16, which can be considered as reactive but rated negative. The reasons for the low titre in those cases can vary and their interpretation has to be done with caution. Those patients might have had contact with Babesia some time ago and the parts of the persisting antibodies can perform some reactivity. This would be supported by the fact that 20 out of the 28 IFAT reactive hunters recalled having a tick bite in the past years. Nevertheless, this hypothesis is rather vague and other options, such as cross-reactivity, unspecific reactivity and differences in individual interpretation by the investigators and laboratories,[12, 15, 19] have to be taken into account. Other detection methods such as PCR are not suitable in this case because in immunocompetent individuals infections due to *B. divergens* or *B. venatorum* are self-limiting and the parasitemia may last for a short period. Only within this time frame, the *Babesia* DNA is detectable by PCR in EDTA blood[22-24]. Therefore, it is no wonder that in our cohort no PCR-positive sample was found, but for the sake of completeness and not to miss an acute infection, this investigation

was additionally performed. Overall, comparing serological studies is problematic as there is still no standardised method for IFAT examinations[1].

Our study shows that healthy individuals were exposed to *B. divergens* or *B. venatorum* with a prevalence rate of 0.12%. Our results differ from a study in Germany, which shows a sero-prevalence rate of 3.6% to *B. divergens*[19]. This prevalence rate in Germany was because these subjects were exposed to more tick bites than healthy blood donors examined in this study[19, 12].

Our prevalence rate of 0.12% in healthy subjects (anti-*Babesia*-antibodies to *B. divergens* / *B. venatorum*) differ too from the first study in Tyrol, which showed a sero-prevalence rate of 2.1% IgG antibodies against *B. divergens* in healthy blood donors[15]. In contrast, a study from Slovenia,[20] which was also carried out in spring, and a study from France,[21] which was carried out from December to December, showed sero-prevalence of approximately 10% and 0.1% of *B. divergens* in foresters who spend much work-related time outdoors.

This suggests a much higher prevalence in Slovenia as compared to our data. An explanation of these various results could be the use of non-standardized IFA tests[1] since the vector *I. ricinus* is also the most widespread tick species in Slovenia[20] with the same pathogens as in Austria[9-11]. In contrast, in France the risk of infection seems to be close to zero and can be correlated with our study. Furthermore, the investigations on *B*. *divergens* were carried out in northeastern France only in forest workers who had a proven tick contact by other tick pathogens[21].

These highly diverse results highlight the importance of those screenings to assess the risk posed by this type of zoonotic infections. In addition, due to the lack of long-term follow-up findings for *B. divergens* and *B. venatorum*, we do not know how fast antibody titres decrease over time after infection. The collection of blood samples for our study was performed from March to April, when tick activity is low[5], and is the first study to be conducted in Austria during this period. Given the titre level of 1:64 in our research, we assume that this tested hunter had a recent tick bite before winter.

On the district level, in Imst, evidence of *Babesia* spp. could be found in 47 out of 48 *I. ricinus* examined at all stages (adult, nymphs and larvae) in a previous study[11]. In our study, 43 active hunters were examined in this district without supporting evidence of *Babesia* spp. In the district of Schwaz (Fügen), 48 *I. ricinus* ticks were examined; no positive result was detected in them[11] or in the 71 surveyed hunters in this district. Recently, a paper was published on screening ticks from the same tick collection used by Blaschitz and colleagues. It is interesting that from this cohort of 2005 in Tyrol only 2 proofs of *B. divergens* were furnished. Schötta and colleagues[25] state that the high prevalence rates, close to 50%,[11] are possibly due to increased collection numbers from the same place, which could reflect the potential of transstadial and transovarial transmission as well as the impact on the development of infection foci[25]. In 2014,

seroprevalence rates of IgG antibodies against *B. divergens* in Tyrol were performed. Kitzbühel is located in the area with an estimation of 1% - 2% and Landeck is located in the area with a seroprevalence less then 1%[15]. In our Study seroprevalence at the district level shows the highest in Kitzbühel with (5.1%, 95% CI: 1-11%) and Landeck with (7.3%, 95% CI: 2-12%). The test person with a titer of 1:64 who was rated positive was also detected in the district Kitzbühel (Table 1). Due to different titer levels and the low prevalence, comparability and interpretation should be considered with great caution.

It is known that *Babesia* spp. occur in wild ungulates, especially in our study area in which the 813 active hunters were screened[28-29]. In regard to the transmission of these tick-borne pathogens spread via routes other than ticks, many questions remain unsolved. In literature the possibility of infection through the handling of killed game, especially wild ungulates, is discussed[30]. Although a high number of the hunters investigated in this study (755, 93%) had regular contact with shot wild ungulates, no evidence of increased risk could be drawn from these results. Therefore, these routes remain rather hypothetical.

In the current study with 813 participants from Tyrol, we assume that one healthy, active hunter had contact with *Babesia* spp. Further evaluation with direct detection of *Babesia* DNA remained negative. This very low prevalence in comparison with other similar studies is interesting and highlights the need for further research. There remains a great concern regarding latent infections that cannot be detected by PCR and show no overt signs of disease. Here, follow-up studies are required to detect possible reactivation. This study was the first in Austria to assess a serological screening in a high-risk population in the early spring when tick activity is still low. We show that anti-*Babesia* antibodies may be detected in an apparently healthy person. The lack of long-term studies on the course of antibodies after exposure to *B. divergens* and *B. venatorum* shows, in addition to the research need mentioned above, a large gap that still needs to be filled. Our study aimed to help clinicians become aware of these zoonotic pathogens to detect possible cases of illness early enough. *Babesia* spp. should be considered in the differential diagnosis of febrile illness occurring after exposure to ticks, particularly in immunocompromised patients.

Ethical statement: Approval for this study was obtained from the Ethics Committee in Salzburg on January 28th, 2015 (No: 415-E/1845/2-2015). The written informed consent was given by the patients for their information to be stored and used for this study.

Conflicts of interest: The authors state that there is no conflict of interest.

Table 1 and Figure 1

district	n (%) examined hunters	n (%) tick contact	n (%) hunting wild ungulates	n (%) (95%CI) IFAT 1:16 reactive results	n (%) IFAT 1:64 reactive results	n (%) PCR reactive results	n (%) hunting wild ungulates IFAT 1:16	n (%) IFAT 1:64
Innsbruck	37 (4,6)	20 (54,1)	28 (75,7)	0 (0,0) (0-0)	0 (0,0)	---------	---------	---------
Innsbruck-Land	98 (12,1)	62 (63,3)	93 (94,9)	1 (1,0) (0-3)	0 (0,0)	0 (0,0)	1	
Imst	43 (5,3)	32 (74,4)	40 (93,0)	1 (2,3) (0-7)	0 (0,0)	0 (0,0)	1	
Kitzbühel*	98 (12,1)	69 (70,4)	94 (95,9)	5 (5,1)* (1-11)	1 (1,0)	0 (0,0)	5	1
Kufstein	123 (15,1)	99 (80,5)	119 (96,7)	2 (1,6) (0-4)	0 (0,0)	0 (0,0)	2	
Landeck	110 (13,5)	59 (53,6)	95 (86,4)	8 (7,3) (2-12)	0 (0,0)	0 (0,0)	8	
Lienz	119 (14,6)	62 (52,1)	115 (96,6)	3 (2,5) (0-5)	0 (0,0)	0 (0,0)	3	
Reutte	114 (14,0)	84 (73,7)	102 (89,5)	5 (4,4) (1-8)	0 (0,0)	0 (0,0)	5	
Schwaz	71 (8,7)	63 (88,7)	69 (97,2)	2 (2,8) (0-7)	0 (0,0)	0 (0,0)	1	
total	813 (100,0)	550 (68,0)	755 (93,0)	27 (3,3)	1 (0,12)	0 (0,0)	26 (92,8)	1 (3,6)

Table 1: Number of examined hunters in the nine Tyrolean districts, their tick contact in recent years, hunting wild ungulates, results in IFAT and PCR investigation. In the district Kitzbühel* the reactive hunter of 1:16 hunter was not listed because it was positive in 1:64. 95%CI: 95% confidence interval

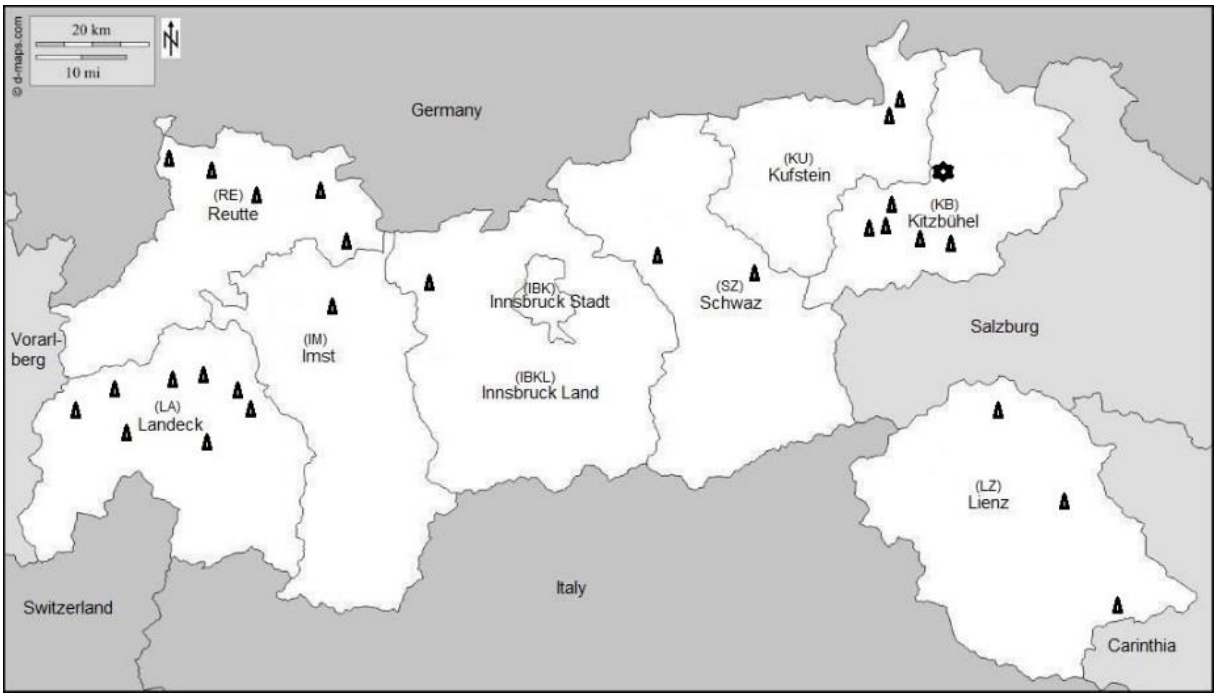

Figure 1: Location of the study area. Light areas show the nine districts of Tyrol (Austria). Geographic proveniences of the hunters with IFAT (anti-*Babesia* antibodies) are indicated by black triangles (titre of 1:16 rate as negative) and a black star (titre of 1:64 rate as positive).

References:

1 Hildebrandt A, Gray JS, Hunfeld KP. Human babesisosis in Eurpoe: what clinicians need to know. *Infection* 2013; 41 (6): 1057-1072.

2 Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA. Babesia: A world emerging. Infect Genet Evol 2012; 12 (8): 1788-1809.

3 Hunfeld KP, Hildebrandt A, Gray JS. Babesiosis: Recent insights into an ancient disease. *Int J Parasitol 2008;* 38 (11): 1219-1237.

4 Carvalho AS, Rodriguez MS, Matthiesen R. Red Blood Cells in Clinical Proteomics. *Methods Mol Biol* 2017; 1619: 173-181.

5 Vannier E, Krause PJ. Human Babesiosis. *N Engl J Med* 2012; 366: 2397-407.

6 Mareedu N, Schotthoefer AM, Tompkins J, Hall MC, Fritsche TR, Frost HM. Risk Factors for Severe Infection, Hospitalization, and Prolonged Antimicrobial Therapy in Patients with Babesiosis. *Am J of Trop Med Hyg 2017;* 97(4): 1218-1225.

7 Burgess MJ, Rosenbaum ER, Pritt BS, Haselow DT, Ferren KM, Alzghoul BN, *et al*. Possible Transfusion-Transmitted Babesia divergens-like/MO-1 Infection in an Arkansas Patient. *Clin Infect Dis* 2017; 64 (11): 1622-1625.

8 Levin AE, Williamson PC, Bloch EM, Clifford J, Cyrus S, Shaz BH, *et al*. Serologic screening of United States blood donors for *Babesia microti* using an investigational enzyme immunoassay. *Transfusion* 2016; 56: 1866-1874.

9 Stanek G. Büchse der Pandora: Krankheitserreger in *Ixodes ricinus*-Zecken in Europa [Pandora's Box: pathogens in *Ixodes ricinus* ticks in Central Europe]. *Wien Klin Wochenschr* 2009; 121: 673-683.

10 Homer MJ, Aguilar-Delfin I, Telford III SR, Krause PJ, Persing DP. Babesiosis. *Clin Microbiol Rev* 2000; 13 (3): 451-469.

11 Blaschitz M, Narodoslavsky-Gföller M, Kanzler M, Stanek G, Walochnik J: *Babesia* species occurring in Austria *Ixodes ricinus* ticks. *Appl Environ Microbiol* 2008; 74

12 Chmielewska-Badora J, Moniuszko A, Żukiewicz-Sobczak W, Zwoliński J, Piątek J, Pancewicz S. Serological survey in persons occupationally exposed to tick-borne pathogens in cases of co-infections with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti*. Ann Agric Environ Med 2012; 19 (2): 271-4.

13 Pańczuk A, Tokarska-Rodak M, Kozioł-Montewka M, Plewik D. The incidence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Babesia microti* coinfections among foresters and farmers in eastern Poland. J Vector Borne Dis 2016; 53 (4): 348-354.

14 Matei IA, Kalmár Z, Lupşe M, D'Amico G, Ionică AM, Dumitrache MO, *et al*. The risk of exposure to rickettsial infections and human granulocytic anaplasmosis associated with *Ixodes ricinus* tick bites in humans in Romania: A multiannual study. *Ticks Tick Borne Dis* 2017; 8 (3): 375-378.

15 Sonnleitner ST, Fritz J, Bednarska M, Baumgartner R, Simeoni J, Zelger R, *et al*. Risk assessment of transfusion-associated babesiosis in Tyrol: appraisal by seroepidemiology and polymerase chain reaction. *Transfusion* 2014; 54: 1725-1732.

16 Blum S, Gattringer R, Haschke E, Walochnik J, Tschurtschenthaler G, Lang F, *et al*. The Case | Hemolysis and acute renal failure. *Kidney Int* 2011; 80: 681–683.

17 Ramharter M, Walochnik J, Lagler H, Winkler S, Wernsdorfer WH, Stoiser B, *et al*. Clinical and molecular characterization of a near fatal case of human babesiosis in Austria. *J Travel Med* 2010; 17 (6): 416-418.

18 Blaschitz M, Narodoslavsky-Gföller M, Kanzler M, Stanek G, Walochnik J. *Babesia* species occurring in Austria *Ixodes ricinus* ticks. *Appl Environ Microbiol* 2008; 74 (15): 4841-4846.

19 Hunfeld KP, Lambert A, Kampen H, Albert S, Epe C, Bradel V, *et al*. Seroprevalence of *Babesia* Infections in Humans Exposed to Ticks in Midwestern Germany. *J Clin Microbiol* 2002; 40 (7): 2431-2436.

20 Rojko T, Duh D, Avsic-Zupanc A, Strle F, Lotric-Furlan S. Seroprevalence of *Babesia divergens* infection among forestry workers in Slovenia. *International Journal of Medical Microbiology* 2008; 298: 347-350.

21 Rigaud E, Jaulhac B, Garcia-Bonnet N, Hunfeld KP, Féménia F, Huet D, *et al*. Seroprevalence of seven pathogens transmitted by the *Ixodes ricinus* tick in forestry workers in France. *Clin Microbiol Infect* 2016; 22: 735.e1-735.e9.

22 Herwaldt B, Cacciò S, Gherlinzoni F, Aspöck H, Slemenda SB, Piccaluga PP, *et al*. Molecular Characterization of a Non–*Babesia divergens* Organism Causing Zoonotic Babesiosis in Europe. *Emerg Infect Dis* 2003; 9 (8): 943-948.

23 Mørch K, Holmaas G, Frolander PS, Kristoffersen EK. Severe human *Babesia divergens* infection in Norway. *Int J Infect Dis* 2015; 33: 37-38.

24 Häselbarth K, Tenter AM, Brade V, Krieger G, Hunfeld KP. First case of human babesiosis in Germany - Clinical presentation and molecular characterisation of the pathogen. *Int J Med Microbiol* 2007; 297 (3): 197-204.

25 Schötta AM, Wijnveld M, Stockinger H, Stanek G. Reverse Line Blot-based Detection Approaches of Microbial Pathogens in *Ixodes ricinus* Ticks Collected in Austria and Impact of the Chosen Method. *Appl Environ Microbiol* 2018; doi:10.1128/AEM.00489-17.

26 Vannier EG, Diuk-Wasser MA, Mamoun CB, Krause PJ. Babesiosis. *Infect Dis Clin North Am* 2015; 29 (2): 357-370.

27 Ord RL, Lobo CA. Human Babesiosis: Pathogen, Prevalence, Diagnosis and Treatment. *Curr Clin Microbiol Rep* 2015; 2 (4): 173-181.

28 Cézanne R, Mrowietz N, Eigner B, Duscher GG, Glawischnig W, Fuehrer HP. Molecular analysis of *Anaplasma phagocytophilum* and *Babesia divergens* in red deer (*Cervus elaphus*) in Western Austria. *Mol Cell Probes* 2017; 31: 55-58.

29 Silaghi C, Hamel D, Pfister K, Rehbein S. *Babesia* species and co-infection with *Anaplasma phagocytophilum* in free-ranging ungulates from Tyrol (Austria). *Wien Tierärztl Mschr – Vet Med Austria* 2011, 98: 268-274.
30 Skotarczak B, Adamska M, Sawczuk M, Maciejewska A, Wodecka B, Rymaszewska A. Coexistence of tick-borne pathogens in game animals and ticks in western Poland. *Veterinarni Medicina* 2008, 53 (12): 668–675.

Zusammenfassung und Ausblick

In dieser Doktorarbeit wurden zoonotische Pathogene in immunkompetenten Personen und in Wildtieren untersucht. Die Ergebnisse dieser Arbeit sind Grundlage für das aktuelle Vorkommen der Erreger. Daraus profitieren sowohl die öffentliche Gesundheit als auch unsere Ärzte und sie dienen der Prävention. Denn eine Früherkennung von Zoonosen durch Überwachung an der Schnittstelle Mensch-Tier ist ein entscheidender Schritt zur Bekämpfung und zur Prävention von Zoonosen (Salyer et al. 2017).

Der im 1. Kapitel untersuchte Erreger *E. multilocularis* ist im Bundesland Tirol endemisch und verursacht die humane AE (Auer 2006). Diese eher seltene aber lebensbedrohliche Infektion (Brunetti et al. 2010) wurde in den letzten Jahren vermehrt diagnostiziert (Schneider et al. 2013). JägerInnen werden in vielen Studien aufgrund ihrer jagdlichen Aktivitäten als Risikogruppe, eine AE zu entwickeln, gesehen (Kern et al. 2003, 2004, Tiaoying et al. 2005, Schneider et al. 2013, Sadkowska-Todys et al. 2015). Trotz des hohen Gefährdungspotentials infolge ihrer langen Jagdaktivitäten (52,6% jagen seit mehr als 20 Jahren) gab es keinen eindeutigen Beweis für die Diagnose einer AE.
Trotz der Tatsache, dass JägerInnen mit *E. multilocularis* vermehrt in Kontakt kommen, gab es bei der Erkrankungshäufigkeit keinen Unterschied zwischen JägerInnen und der nichtjagenden Bevölkerung.
Im 2. Kapitel war das Rehwild (*Capreolus capreolus*) im Fokus unserer Studie. Die bestehende FSME-Verbreitungskarte für Österreich wird anhand von diagnostizierten humanen FSME-Erkrankungen erstellt (Baxter Healthcare GmbH, Wien). Die hohe Durchimpfungsrate der österreichischen Bevölkerung (Donoso Mantke et al. 2011; Heinz et al. 2013; Walder et al. 2008) führt zu einer Verschleierung des FSME-Infektionsrisikos. Es treten zwar weniger Fälle von FSME-Erkrankungen auf, doch die Gefahr einer Infektion bleibt für ungeimpfte Personen bestehen (Stefanoff 2011).
Rehwild ist einem starken Befall des Ektoparasiten *I. ricinus* ausgesetzt (Kiffner et al. 2010; Vor et al. 2011), welcher das FSME-Virus repliziert und durch einen Stich überträgt (Labuda et al. 1999). Das Rehwild selbst entwickelt nach dem Kontakt keine symptomatische FSME-Infektion (Nosek et al. 1967, Radda et al. 1968a, b), jedoch FSME-Antikörper (Gerth et al. 1995; Kiffner et al. 2012; Nosek et al. 1967; Radda et al. 1968a, b; Skarphedinsson et al. 2005) und wird dadurch zu einer sehr guten Indikatorspezies. Unsere Ergebnisse zeigen FSME-Antikörper bei Rehwildseren in Gebieten, in denen noch kein humaner FSME-Erkrankungsfall aufgetreten ist. So konnten wir die These, Rehwild als eine sehr gute Indikatorspezies zu sehen, bestätigen.
Diese Erkenntnis ist besonders für noch nicht ausgewiesene Gebiete wichtig. So kann die FSME Risikokarte in Österreich komplettiert werden. Gleichbedeutend ist sie für Gebiete mit

hoher Durchimpfungsrate in der Bevölkerung, um das potentielle Infektionsrisiko aufzuzeigen. Infektionskrankheiten, wie etwa im 2. Kapitel beschrieben oder auch die bekannte Lyme Borreliose, die in Österreich auf annähernd 50 000 Fälle pro Jahr geschätzt wird (Stanek 2009) zeigen den extremen Kontakt von Menschen zu Zecken.

Durch das häufige Vorkommen der vorher genannten Erreger haben Ärzte ein Bewusstsein für diese zoonotischen Pathogene entwickelt. Hingegen sind *Babesien* spp., die im 3. Kapitel behandelt werden, in Österreich noch wenig allgemein bekannt (Sonnleitner et al. 2014). Untersuchungen von *Babesien* spp. Erreger sind besonders wichtig. *Babesien* spp. sind Hämoprotozoen (Schnittger et al. 2012) und unterscheiden sich in der Behandlung von bakteriell übertragenen Zeckenpathogenen essentiell (Sanchez et al. 2016; Kletsova et al. 2017).

Unsere Forschungsergebnisse zeigen einen einzigen positiven Nachweis mittels einem anti-*Babesien*-Antikörper Titer von 1:64. 27 Probanden zeigen einen grenzwertigen anti-*Babesien*-Antikörper mit einer Titerhöhe von 1:16.

Die Abwesenheit von DNA lässt auf eine Selbstlimitierung immunkompetenter Personen schließen.

In den Bereichen “zoonotische Pathogene in Österreich“ sind noch viele Lücken zu schließen. In 1. Kapitel konnten wir JägerInnen als Risikogruppe ausschließen. Jedoch erklärt dies nicht die vermehrt aufgetretenen humanen AE Erkrankungsfälle in Österreich. Daraus ergibt sich ein enormer Forschungsbedarf, zum Beispiel neue potentielle Übertragungswege aufzuzeigen. *E. multilocularis* zirkuliert hauptsächlich in einem sylvatischen Lebenszyklus, indem der Rotfuchs (*Vulpes vulpes*) als Haupt- und Endwirt und Nagetiere wie z.B. *Microtus arvalis, Arvicola terrestris, Ondatra zibethica* als Zwischenwirte fungieren. (Eckert et al. 2001, Oksanen et al. 2016).

Bedeutungsvoll, aber viel zu wenig bekannt ist der synantrope Lebenszyklus der Rotfüchse. Sie adaptieren sich durch ihre hohe Anpassungsfähigkeit immer öfters in Städten (Gloor et al. 2001, Romig et al 2006, Robardet et al. 2008). Damit findet eine Kontamination der urbanen Umwelt mit *E. multilocularis* statt (Deplazes et al. 2004).

Vorliegende Forschungsergebnisse zeigen, dass Haustiere, insbesondere Hunde, als Endwirte fungieren und so die Umwelt kontaminieren, indem sie infektiöse *E. multilocularis*-Eier ausscheiden (Eckert et al. 2004, Kern et al. 2004, Duscher et al. 2015).

Interessant und wichtig wäre eine Beprobung von Haustieren und von Zwischenwirten um aktuelle Prävalenzraten zu erhalten. So bekäme man Informationen über eine potentielle Gefährdung, die von Haustieren ausgeht.

Grundlegend wichtig ist die Aufklärung der Bevölkerung, sowohl in ruralen als auch in urbanen Gebieten, um ein Umdenken zu schaffen und so präventiv dem Erreger *E. multilocularis* entgegenzuwirken.

Die Forschungsergebnisse im 2. Kapitel sollen einen Beitrag für die Bevölkerung leisten und ihr nahe bringen, dass Infektionen auch in Gebieten möglich sind, die in der derzeitigen FSME Risikokarte nicht ausgewiesen sind. Dadurch soll auch das Bewusstsein für diesen gefährlichen Erreger in der Bevölkerung gestärkt werden und einen Anreiz bieten die hohe Durchimpfungsrate (Donoso Mantke et al. 2011; Heinz et al. 2013; Walder et al. 2008) in Österreich beizubehalten bzw. zu steigern.

Obwohl sich anhand unserer Ergebnisse - wie im Kapitel 3 beschrieben - nur ein einziger positiver Nachweis von *Babesia* spp. erbringen lässt, folgt daraus, dass eine potentielle Infektion mit *Babesien* spp. jederzeit möglich ist.

Es handelt sich bei unseren Untersuchungen erst um die zweite Screeninguntersuchung von Babesien in Österreich und um das erste serologische *in vivo* Screening, das im Frühjahr durchgeführt wurde, in der die Zeckenaktivität noch gering ist. Daraus folgt, dass mangels dieser Informationen über *Babesia* spp. vorwiegend bakterielle Infektionen nach einem Zeckenstich in Betracht gezogen werden (Stanek 2009, Sonnleitner et al. 2014) obwohl Koinfektionen weit verbreitet sind (Heyman et al. 2010; Lommano et al. 2012; Chmielewska-Badora et al. 2012; Pańczuk et al. 2016).

Unsere Forschungsergebnisse sollen vor allem praktizierenden Ärzten als Information dienen, für immunsupprimierte und splenektomierte Personen ist diese Infektion lebensbedrohlich (Krause et al. 2003, Leiby 2011, Mareedu et al. 2017).

Um weitere wichtige Erkenntnisse über die Epidemiologie von *Babesia* spp in Österreich zu erhalten, sollten nach jedem Zeckenstich Untersuchungen betreffend *Babesien* spp. durchgeführt werden.

Untersucht werden sollten möglichst bald auch Blutprodukte wegen der Gefahr bei Übertragung derselben. Amerikanische Studien verzeichnen einen steigenden Trend dieser Übertragung durch den Erreger *B. microti* (Bloch et al. 2016; Levin et al. 2016). In Österreich ist diese potentielle Möglichkeit der Übertragung noch viel zu wenig erforscht (Sonnleitner et al. 2014), obwohl in den letzten Jahren schon autochthone Infektionen durch diesen Erreger in Europa aufgetreten sind (Hildebrandt et al. 2007, Arsuaga et al. 2016).

Es besteht ein enormer Forschungsbedarf um mögliche Infektionen durch Blutprodukte zu vermeiden. Nur durch so gewonnenen Daten und neuen Erkenntnissen kann man einer potentiellen Gefahr einer Infektion entgegenwirken.

Literaturverzeichnis

Achazi, K., Růžek, D., Donoso-Mantke, O., Schlegel, M., Ali, H. S., Wenk, M., Schmidt-Chanasit, J., Ohlmeyer, L., Rühe, F., Vor, T., Kiffner, C., Kallies, R., Ulrich, R. G., Niedrig, M. (2011). Rodents as sentinels for the prevalence of tick-borne encephalitis virus. Vector Borne Zoonotic Diseases. 11: 641-647

Agergaard, C. N., Rosenstierne, M. W., Bødker, R., Rasmussen, M., Andersen, P. H. S., Fomsgaard, A. (2019). New tick-borne encephalitis virus hot spot in Northern Zealand, Denmark, October 2019. Euro Surveillance. 24, 43

Ahmed, S., Dávila, J. D., Allen, A., Haklay, M., Tacoli, C., Fèvre, E. M. (2019). Does urbanization make emergence of zoonosis more likely? Evidence, myths and gaps. Environment & Urbanisation. 31, 2: 443-460

Arsuaga, M., Gonzalez, L. M., Lobo, C. A., de la Calle, F., Bautista, J. M., Azca´rate, I. G., Puente, S., Montero, E. (2016). First Report of Babesia microti-Caused Babesiosis in Spain. *Vector-Borne and Zoonotic* Diseases. 16, 10: 677-679

Auer, H. (2006). Die Bedeutung labordiagnostischer Untersuchungen für die Klinik, die Epidemiologie und Prävention der alveolären Echinokokkose – Erfahrungen zweier Jahrzehnte in Österreich. [Relevance of parasitological examinations for the clinical course, epidemiology and prevention of alveolar echinococcosis – experiences of more than two decades in Austria]. Wiener Klinische Wochenschrift. 118 (19-20 Suppl 3): 18-26

Auer, H., Aspöck H. (2001): Human alveolar echinococcosis and cystic echinococcosis in Austria: The recent epidemiological situation. Helminthologia.38: 3-14

Belova, O. A., Burenkova, L. A., Karganova, G. G. (2012). Different tick-borne encephalitis virus (TBEV) prevalences in unfed versus partially engorged ixodid ticks – evidence of virus replication and changes in tick behavior. Ticks and Tick-borne Diseases. 3: 240-246

Blaschitz, M., Narodoslavsky-Gföller, M., Kanzler, M., Stanek, G., Walochnik, J. (2008). *Babesia* species occurring in Austria *Ixodes ricinus* ticks. Applied and Environmental Microbiology. 74,15: 4841-4846

Bloch, E. M., Levin, A. E., Williamson, P. C., Cyrus, S., Shaz, B. H., Kessler, D., Gorlin, J., Bruhn,R., Tzong-Hae Lee, Montalvo, L., Kamel, H., Busch, M. P. (2016). A prospective evaluation of chronic *Babesia microti* infection in seroreactive blood donors. Transfusion. 56: 1875-1882

Brockmann, S. O., Oehme, R., Buckenmaier, T.,Beer, M., Jeffery-Smith, A., Spannenkrebs, M., Haag-Milz, S., Wagner-Wiening, C., Schlegel, C., Fritz, J., Zange, S., Bestehorn, M., Lindau, A., Hoffmann, D., Tiberi, S., Mackenstedt, U., Doble, G. (2018). A cluster of two human cases of tick-borne encephalitis (TBE) transmitted by unpasteurised goat milk and cheese in Germany, May 2016. Euro Survillance. 23, 15

Brunetti, E., Kern, P., Vuitton, D.A., Writing Panel for the WHO-IWGE. (2010). Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Tropica. 114: 1-16

Carvalho, A. S., Rodriguez, M. S., Matthiesen, R. (2017). Red Blood Cells in Clinical Proteomics. Methods in Molecular Biology. 1619: 173-181

CDC Centers for Disease Control and Prevention 24/7 (2018). Zoonotic Disease. https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html [abgefragt 06/2018]

Cézanne, R., Mrowietz, N., Eigner, B., Duscher, G. G., Glawischnig, W., Fuehrer, H. P. (2017). Molecular analysis of *Anaplasma phagocytophilum* and *Babesia divergens* in red deer (*Cervus elaphus*) in Western Austria. Molecular and Cellular Probes. 31: 55-58

Chmielewska-Badora, J., Moniuszko, A., Żukiewicz-Sobczak, W., Zwoliński, J., Piątek, J., Pancewicz, S. (2012). Serological survey in persons occupationally exposed to tick-borne pathogens in cases of co-infections with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Babesia microti*. Annals of Agricultural and Environmental Medicine.19, 2: 271-274

Chouhan, M. D., Wiley, E., Chiodini, P. L., Amin, Z. (2019). Hepatic alveolar hydatid disease (*Echinococcus multilocularis*), a mimic of liver malignancy: a review for the radiologist in non-endemic areas. Clinical Radiology. 74, 4: 247-256

Cull, B., Pietzsch, M. E., Gillingham, E. L., McGinley, L., Medlock, J. M., Hansford, K. M. (2019). Seasonality and anatomical location of human tick bites in the United Kingdom. Zoonoses and Public Health. https://doi.org/10.1111/zph.12659

Deplazes, P., van Knapen, F., Schweiger, A., Overgaauw, p. a. (2011). Role of pet dogs and cats in the transmission of helminthic zoonoses in Europe, with a focus on echinococcosis and toxocarosis. Veterinary Parasitology. 182, 1: 41-53

Deutz, A., Fuchs, K., Schuller, W., Nowotny, N., Auer, H., Aspöck, H., Stünzner, D., Kerbl, U., Klement, C., Köfer, J. (2003). Seroepidemiologische Untersuchung von Jägern in Südostösterreich – Prävalenzen, Risikopotentiale und Vorbeugemaßnahmen [Seroepidemiological studies of zoonotic infections in hunters in southeastern Austria – prevalences, risk factors and preventive methods]. Berliner Und Munchener Tierarztliche Wochenschrift. 116, 7-8: 306–11

Dobler, G., Fingerle, V., M. Hagedorn, P., Pfeffer, M., Silaghi, C., Tomaso, H., Henning, K., Niedrig, M. (2014). Gefahren der Übertragung von Krankheitserregern durch Schildzecken in Deutschland. [Threat of transmission of infectious pathogens by *Ixodes ricinus* ticks in Germany], Bundesgesundheitsblatt. 57: 541–548

Dobler, G., Hufert, F. T., Pfeffer, M., Essbauer, S. (2011). Tick-borne encephalitis: from microfocus to human disease. Progress in Parasitology. Parasitology Research Monographs. 2: 323-331

Donoso Mantke, O., Escadafal, C., Niedrig, M., Pfeffer, M. (2011). Working Group for Tick-Borne Encephalitis Virus C, Tick-borne encephalitis in Europe, 2007 to 2009. Euro Surveillance. Europe`s journal on infectious disease surveillance epidemiology, prevention and control. DOI:10.2807/ese.16.39.19976-en

Duscher, G.G., Leschnik, M., Fuehrer, H.P., Joachim, A. (2015). Wildlife reservoirs for vector-borne canine, feline and zoonotic infections in Austria. International Journal for Parasitology: Parasite and Wildlife. 4: 88-96.

Duscher, T., Hodžić, A., Glawischnig, W., Duscher, G.G. (2017). The raccoon dog (*Nyctereutes procyonoides*) and the raccoon (*Procyon lotor*) – their role and impact of maintaining and transmitting zoonotic diseases in Austria, Central Europe. Parasitology Research. 116: 1411-1416

ECDC, European Centre for Disease Prevention and Control. Annual epidemiological report 2018 – tick borne encephalitis. Stockholm [03/2018]

Eckert, J. (1996). *Echinococcus multilocularis* in Europa – Versuch einer Risikobewertung. [Echinococcus multilocularis in Europe: an attempt to assess the risk]. Mitteilungen der Österreichischen Gesellschaft für Tropenmedizin und Parasitologie. 18: 131-140

Eckert, J., Deplazes, P. (2004). Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clinical Microbiology Review. 17, 1: 107–35

Eckert, J., Deplazes, P., Craig, P.S., Gemmell, M.A., Gottstein, B., Heath, D., Jenkins, D.J., Kamiya, M., Lightowlers, M. (2001). Echinococcosis in animals: Clinical aspects, diagnosis and treatment. In: Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, Z. (eds.) WHO/OIE Manual on Echinococcosis in Humans and Animals. 73–99

Esser, H. J., Mögling, R., Cleton, N. B., van der Jeugd, H., Sprong, H., Stroo, A., Koopmans, M. P. G., de Boer, W. F., Reusken, C. B. E. M. (2019). Risk factors associated with sustained circulation of six zoonotic arboviruses: a systematic review for selection of surveillance sites in non-endemic areas. Parasites & Vectors. 12. 265

Estrada-Peña, A., de la Fuente, J. (2014). The ecology of ticks and epidemiology of tick-borne viral diseases. Antiviral Research. 108C: 104-128

Gabrielli, S., Calderini, P., Cassini, R., Galuppi, R., Tampieri, M. P., Pietrobelli, M., Cancrini, G. (2014): Human exposure to piroplasms in Central and Northern Italy. Veterinaria Italiana. 50, 1: 41-47

Gäumann, R., Mühlemann, K., Strasser, M., Beuret, C. M. (2010). High-throughput procedure for tick surveys of tick-borne encephalitis virus and its application in a national surveillance study in Switzerland. Applied and Environmental Microbiology76: 4241-4249

Gerth, H. J., Grimshandl, D., Stage, B., Doller, G., Kunz, C. (1995). Roe deer as sentinels for endemicity of tick-borne encephalitis virus. Epidemiology and Infection. 115: 355-365

GfK Healthcare: Impfstatus 2014. Frühsommer-Meningo-Enzephalitis (FSME) und FSME-Schutzimpfung in Österreich: Update 2014. Wiener Medizinische Wochenschrift: Ausgabe 13-14/2015

Gherman, C. M., Mihalca, A. D. (2017). A synoptic overview of golden jackal parasites reveals high diversity of species. Parasites & Vectors. 10: 419

Gloor, S., Bontadina, F., Heggun, D., Deplazes, P., Breitenmoser, U. (2001). The rise of urban fox populations in Switzerland. Mammalian Biology. 66: 155-164

Häselbarth, K., Tenter, A. M., Brade, V., Krieger, G., Hunfeld, K. P. (2007). First case of human babesiosis in Germany - Clinical presentation and molecular characterisation of the pathogen. International Journal of Medical Microbiology. 297, 3: 197-204

Heinz, F. X., Stiasny, K., Holzmann, H., Grgic-Vitek, M., Kriz, B., Essl, A., Kundi, M. (2013). Vaccination and tick-borne encephalitis, central Europe. Emerging Infectious Diseases. 19: 69-76

Herwaldt, B., Cacciò, S., Gherlinzoni, F., Aspöck, H., Slemenda, S. B., Piccaluga, P. P., Martinelli, G., Edelhofer, R., Hollenstein, U., Poletti, G., Pampiglione, S., Löschenberger,K., Tura, S., Pieniazek, N. (2003). Molecular Characterization of a Non–*Babesia divergens*

Organism Causing Zoonotic Babesiosis in Europe. Emerging Infectious Diseases. 9, 8: 943-948

Heyman, P., Cochez, C., Hofhuis, A., van der Giessen, J., Sprong, H., Porter, S. R., Losson, B., Saegermann, C., Donoso-Mantke, O., Niedrig, M., Papa, A. (2010). A clear and present danger: tick-borne diseases in Europe. Expert Review of Anti-infective Therapy. 8, 1: 33-50

Hildebrandt, H., Hunfeld, K. P., Baier, M. (2007). First confirmed autochthonous case of human Babesia microti infection in Europe. European Journal of Clinical Microbiology & Infectious Diseases. 26: 595-601

Holbach, M., Oehme, R. (2002). FSME und Lyme-Borreliose. Erregerverbreitung in Zecken und Erkrankungsrisiken in einem FSME-Risikogebiet. Fortschritte der Medizin. Originalien. 120: 113-118.

Holzmann, H., Aberle, S. W., Stiasny, K., Werner, P.,Mischak, A., Zainer, B.,Netzer, M., Koppi, S., Bechter, E., Heinz, F. X. (2009). Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. Emerging Infectious Diseases. 15: 1761-1763

Homer, M. J., Aguilar-Delfin, I., Telford III, S. R., Krause, P. J., Persing, D. H. (2000). Babesiosis. Clinical Microbiology Reviews. 13, 3: 451-469

Horst, H. (1991). Einheimische Zeckenborreliose (Lyme-Krankheit) bei Mensch und Tier. Perimed Fachbuch-Verlagsgesellschaft mbH, D-8520 Erlangen, 200 S

Hunfeld, K. P., Hildebrandt, A., Gray, J. S. (2008). Babesiosis: Recent insights into an ancient disease. International Journal of Parasitology. 38, 11: 1219-1237

Jeppesen, J. (1990). Home range and movements of free-ranging roe deer (*Capreolus capreolus*) at Kalø. Danish review of game biology. 14: 2-14

Kayser, F. H., Bienz, K. A., Eckert, L., Lindenmann, L. (1993). Medizinische Mikrobiologie, Immunologie, Bakteriologie, Mykologie, Virologie, Parasitologie. Georg Thieme Verlag, D-7000 Stuttgart, 604 S

Karamon, J., Sroka, J., Dąbrowska, J., Bilska-Zając, E., Zdybel, J., Kochanowski, M.,Różycki, M., Cencek, T. (2019). First report of *Echinococcus multilocularis* in cats in Poland: a monitoring study in cats and dogs from a rural area and animal shelter in a highly endemic region. Parasites & Vectors. 12, 313

Kern, P., Ammon, A., Kron, M., Sinn, G., Sander, S., Petersen, L.R., Gaus, W., Kern, P. (2004). Risk factors for alveolar echinococcosis in humans. Emerging Infectious Diseases. 10: 2088-209

Kern, P., Bardonnet, K., Renner, E., Auer, H., Pawlowski, Z., Ammann, R.W., Vuitton, D. A. (2003). European echinococcosis registry: Human alveolar echinococcosis, Europe, 1982-2000. Emerging Infectious Diseases. 9: 343-349

Kiffner, C., Lödige, C., Alings, M., Vor, T., Rühe, F. (2010). Abundance estimation of Ixodes ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). Experimental and Applied Acarology. 52: 73-84

Kiffner, C., Lödige, C., Alings, M., Vor, T., Rühe, F. (2011). Attachment site selection of ticks on roe deer, *Capreolus capreolus*. Experimental and Applied Acarology. 53: 79-94

Kiffner, C., Vor, T., Hagedorn, P., Niedrig, M., Rühe, F. (2012). Determinants of tick-borne encephalitis virus antibody presence in roe deer (*Capreolus capreolus*) sera. Medical and Veterinary Entomology. 26: 18-25

Kletsova, E. A., Spitzer, E. D., Fries, B. C., Marcos, L. A. (2017). Babesiosis in Long Island: review of 62 cases focusing on treatment with azithromycin and atovaquone. Annals of Clinical Microbiology and Antimicrobials. 16, 26: 2-7

Kmetiuk, L. B., Krawczak, F. S., Machado, F. P., Paploski, I. A. D., Martins, T. F., Teider-Junior, P. I., et al. (2019). Ticks and serosurvey of anti-*Rickettsia* spp. antibodies in wild boars (*Sus scrofa*), hunting dogs and hunters of Brazil. PLoS Neglected Tropical Diseases. 13, 5: e0007405

Kotwa, J. D., Isaksson, M., Jardine, C. M., Campbell, G. D., Berke, O., Pearl, D. L., Mercer, N. J., Osterman-Lind, E., Peregrine, A. S. (2019). *Echinococcus multilocularis* Infection, Southern Ontario, Canada. Emerging Infectious Diseases. 25, 2: 265-272

Krause, P. J., McKay, K., Gadbaw, J., Christianson, D., Closter, L., Lepore, T., Telford, S. R., Sikand, V., Ryan, R., Persing, D., Radolf, J. D., Spielman, A., Tick-Borne Infection Study Group. (2003). Increasing health burden of human babesiosis in endemic sites. The American Journal of Tropical Medicine and Hygiene. 68:431–436

Krause, P. J., Spielman, A., Telford III, S. R., Sikand, V. K., McKay, K., Christianson, D., Pollack, R. J., Brassard, P., Magera, J., Ryan, R., Persing, D. H. (1998). Persistent parasitemia after acute babesiosis. The New England Journal of Medicine. 339: 160-165

Kreidl, P., Allerberger, F., Judmaier, G., Auer, H., Aspöck, H., Hall, A.J. (1998). Domestic pets as risk factors for alveolar hydatid disease in Austria. American Journal of Epidemiology. 147: 978-981

Kunze, U. (2016). The international scientific working group on tick-borne encephalitis (ISW TBE): review of 17 years of activity and commitment. Ticks and Tick-borne Diseases. 7, 3:399–404

Kuehn, B. (2019). Tuberculosis in deer hunters. Journal of the American Medical Association. 322, 18: 1757

Kupča, A. M., Essbauer, S., Zoeller, G., de Mendonça, P. G., Brey, R., Rinder, M., Pfister, K., Spiegel, M., Doerrbecker, B., Pfeffer, M., Dobler, G. (2010). Isolation and molecular characterization of a tick-borne encephalitis virus strain from a new tick-borne encephalitis focus with severe cases in Bavaria, Germany. Ticks and Tick-borne Diseases. 1: 44-51

Labuda, M., Randolph, S. E. (1999). Survival strategy of tick-borne encephalitis virus: Cellular basis and environmental determinants. Zentralblatt Bakteriologie. 289, 5-7: 513-524

Leiby, D. A. (2011). Transfusion-Transmitted Babesia spp.: Bull's-Eye on Babesia microti. Clinical Microbiology Reviews. 24, 1: 14–28

Leiby, D.A., Chung, A. P., Gill, J. E., Houghton, R. L., Persing, D. H., Badon, S., Cable, R. G. (2005). Demonstrable parasitemia among Connecticut blood donors with antibodies to *Babesia microti*. Transfusion. 45, 11: 1804-1810

Levin, A. E., Williamson, P. C., Bloch, E. M., Clifford,Shaz, B. H., Kessler, D., Gorlin, J., Erwin, J. L., Krueger, N. X., Williams, G. V., Penezina, O., Telford IV, S. R., Branda, J. A., Krause, P. J., Wormser, G. P., Schotthoefer, A. M., Fritsche, T. R., Busch, M. P. (2016). Serologic

screening of United States blood donors for *Babesia microti* using an investigational enzyme immunoassay. Transfusion. 56: 1866-1874

Lommano, E., Bertaiola, L., Dupasquier, C., Gern, L. (2012). Infections and Coinfections of Questing *Ixodes ricinus* Ticks by Emerging Zoonotic Pathogens in Western Switzerland. Applied and. Environmental Microbiology. 78, 13: 4606-4612

Lötsch, F., Waneck, F., Auer, H., Kaczirek, K., Karanika, G., Ramharter, M. (2017). FDG-PET/MRI n alveolar echinococcosis. International Journal of Infection Diseases. 64: 67-68

Macpherson, C. N. L. (2005). Human behaviour and the epidemiology of parasitic zoonoses. International Journal of Parasitology. 35, 11-12: 1319-1331

Mareedu, N., Schotthoefer, A. M., Tompkins, J., Hall, M.C., Fritsche, T.R., Frost, H.M. (2017). Risk Factors for Severe Infection, Hospitalization, and Prolonged Antimicrobial Therapy in Patients with Babesiosis. The American Journal of Tropical Medicine and Hygiene. 97, 4: 1218-1225

Michalczyk, M., Sokół, R., Gałęcki, R. (2019). Internal parasites infecting dogs in rural areas. Annals of Parasitology. 65, 2. 151-158

Morand, S., McIntyre, K. M., Baylis, M. (2014). Domesticated animals and human infectious diseases of zoonotic origins: domestication time matters. Infection, Genetics and Evolution. 24: 76-81

Mørch, K., Holmaas, G., Frolander, P. S., Kristoffersen, E. K. (2015). Severe human *Babesia divergens* infection in Norway. International Journal of Infectious Disease. 33: 37-38

Moritz, E. D., Winton, C. S., Tonnetti, L., Townsend, R. L., Berardi, V.P., Hewins, M. E., Weeks, K. E., Dodd, R. Y., Stramer, S. L. (2016). Screening for *Babesia microti* in the U.S. Blood Supply. The New England Journal of Medicine. 375: 2236-2245

Murphy, F. A. (1998). Emerging zoonoses. Emerging Infectious Diseases. 4: 429-435

Nagy, A., Ziadniov, I., Schweiger, A., Schnyder, M., Deplazes, P. (2011). Fellkontamination mit Eiern von zoonotischen Helminthen bei Hof- und Haushunden sowie bei Füchsen. [Hair coat contamination with zoonotic helminth eggs of farm and pet dogs and foxes]. Berliner Und Munchener Tierarztliche Wochenschrift. 124, 11-12: 503-511

Nah, K., Magpantay, F. M. G., Bede-Fazekas, Á., Röst, G., Trájer, A. J., Wu, W., Zhang, X., Wu, J. (2019). Assessing systemic and non-systemic transmission risk of tick-borne encephalitis virus in Hungary. PloS One 14, 6: e0217206

Nardoni, S., Rocchigiani, G., Varvaro, I., Altomonte, I., Ceccherelli, R., Mancianti, F. (2019). Serological and Molecular Investigation on Toxoplasma gondii Infection in Wild Birds. Pathogens, 8: 1-6

Natarajan, P., Miller, A. (2017). Recreational Infections. Infectious Diseases (Fourth Edition) 1: 643-646

Nosek, J., Kozuch, O., Ernek, E., Lichard, M. (1967). Übertragung des Zeckenencephalitis-Virus (TBE) duch die Weibchen von *Ixodes ricinus* und Nymphen von *Haemaphysalis inermis* auf die Rehkitzen (*Capreolus capreolus*). Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 203: 162-166

Oksanen, A., Siles-Lucas, M., Karamon, J., Possenti, A., Conraths, F.J., Romig, T., Wysocki, P., Mannocci, A., Mipratini, D., La Torre, G., Boufana, B., Casulli, A. (2016). The geographical distribution and prevalence of *Echinococcus multilocularis* in animals in the European Union and adjacent countries: a systematic review and meta-analysis. Parasites & Vectors. 9: 519

Pańczuk, A., Tokarska-Rodak, M., Kozioł-Montewka, M., Plewik, D. (2016). The incidence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Babesia microti* coinfections among foresters and farmers in eastern Poland. Journal of Vector Borne Diseases. 53, 4: 348-354

Pańczuk, A., Tokarska-Rodak, M., Plewik, D., Paszkiewicz, J. (2019). Tick exposure and prevalence of *Borrelia Burgdorferi* antibodies among hunters and other individuals exposed to vector ticks in eastern Poland. Roczniki Państwowego Zakładu Higieny. 70, 2:161-168

Petrovic, J. M., Prodanov-Radulovic, J. Z., Mirceta, J.D. (2019). Wild boar meat safety. IOP Conf. Ser.: Earth and Environmental Science. 333 012015

Piarroux, M., Piarroux, R., Giorgi, R., Knapp, J., Bardonnet, K., Sudre, B., Watelet, J., Gerard, A., Beytout, J., Abergel, A., Mantion, G., Vuitton, D. A., Bresson-Hadni, S. (2011): Clinical features and evolution of alveolar echinococcosis in France from 1982 to 2007: Results of a survey in 387 patients. Journal of Hepatology. 55: 1025-1033

Radda, A., Hofmann, H., Kozuch, O., Kunz, C., Nosek, J., Zukrigl, K. (1971). Ein Naturherd des Frühsommer-Meningoenzephalitis-Virus bei St. Florian. Anzeiger für Schädlingskunde und Pflanzenschutz. 44: 96-96

Radda, A., Hofmann, H., Kunz, C. (1968a). Experimentelle Infektion einiger heimischer Saugerarten mit dem Frühsommer-Meningo-Enzephalitis (FSME)-Virus. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 208: 100-104

Radda, A., Kunz, C., Hofmann, H. (1968b). Nachweis von Antikörpern in Wildseren zur Erfassung von Herden des Virus der Frühsommer-Meningo-Enzephalitis FSME) in Niederösterreich. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 208: 88-93

Randolph, S. E. (2009). Tick-borne disease systems emerge from the shadows: the beauty lies in molecular detail, the message in epidemiology. Parasitology. 136: 1403-1413

Reimoser, S., Partl, E., Reimoser, F., Vospernik, V. (2009). Roe-deer habitat suitability and predisposition of forest to browsing damage in its dependence on forest growth—Model sensitivity in an alpine forest region. Ecological Modelling. 220: 2231-2243

Ricken, F.J., Nell, J., Grüner, B., Schmidberger, J., Kaltenbach, T., Kratzer, W., Hillenbrand, A., Henne-Bruns, D., Deplazes, P., Moller, P., Kern, P., Barth, T.F.E. (2017). Albendazole increase the inflammatory response and the amount of Em2-positive small particles of *Echinococcus multilocularis* /spems) in human hepatic alveolar echinococcosis lesions. PLoS Neglected Tropical Disesase. 11, 5: 1-12

Rieille, N., Bressanelli, S., Freire, C. C. M., Arcioni, S., Gern, L., Péter, O., Voordouw, M. J. (2014). Prevalence and phylogenetic analysis of tick-borne encephalitis virus (TBEV) in field-collected ticks (*Ixodes ricinus*) in southern Switzerland. Parasites & Vectors. 7: 443

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Špitalska, E., Kazimírová, M. (2014). *Ixodes ricinus* and ist transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. Frontiers in Public Health. 2, 251: 1-26

Robardet, E., Giraudoux, P., Caillot, C., Boue, F., Cliquet, F., Augot, D., Barrat, J. (2008). Infection of foxes by Echinococcus multilocularis in urban and suburban areas of Nancy, France: influence of feeding habits and environment. Parasite. 15: 77-85

Robertson, D. L., Babin, L. M., Krall, J. R., von Fricken, M. E., Baghi, H., Jacobsen, K. H. (2019). The association between hunter-killed deer and lyme disease in New Jersey, 2000-2014. EcoHealth. 16, 2: 330-337

Romig, T., Dinkel, A., Mackenstedt, U. (2006). The present situation of echinococcosis in Europe. Parasitology International. 55: 187-191

Sadkowska-Todys, M., Baumann-Popczyk, A., Wnukowska, N., Popczyk, B., Kucharczyk, B., Golab, E. (2015). Occurrence and prevalence of selected zoonotic agents: Echinococcus multilocularis, Trichinella spiralis and hepatitis E virus (HEV) in the population of Polish hunters – results of the study conducted in 2010-2012. Przegląd epidemiologiczny. 69, 4: 673-8, 823–7

Salyer, S. J., Silver, R., Simone, K., Barton Behravesh, C. (2017). Prioritizing Zoonoses for Global Health Capacity Building - Themes from One Health Zoonotic Disease Workshops in 7 Countries, 2014–2016. Emerging Infectious Diseases. 23: 57-64

Sanchez, E., Vannier, E., Wormser, G. P., Hu, L. T. (2016). Diagnosis, Treatment, and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis. JAMA, The Journal of the American Medical Association. 315, 16: 1767-77

Schmidt, K., Dressel, K. M., Niedrig, M., Mertens, M., Schüle, S. A., Groschup, M. H. (2013). Public health and vector-borne diseases - a new concept for risk governance. Zoonoses and Public Health. 60, 8: 528-538

Schneider, R., Aspöck, H., Auer, H. (2013). Unexpected increase of alveolar echinococcosis, Austria, 2011. Emerging Infectious Diseases. 19: 475-477

Schnittger, L., Rodriguez, A. E., Florin-Christensen, M., Morrison, D. A. (2012). Babesia: A world emerging. Infection, Genetics and Evolution. 12, 8: 1788-1809

Schötta, A. M., Wijnveld, M., Stockinger, H., Stanek, G. (2017). Approaches for Reverse Line Blot-Based Detection of Microbial Pathogens in *Ixodes ricinus* Ticks Collected in Austria and Impact of the Chosen Method. Applied and Environmental Microbiology. 83: 1-18

Silaghi, C., Hamel, D., Pfister, K., Rehbein, S. (2011). *Babesia* species and co-infection with *Anaplasma phagocytophilum* in free-ranging ungulates from Tyrol (Austria). Wiener tierärztliche Monatsschrift – Veterinary Medicine Austria. 98: 268-274

Sixl, W., Nosek, J. (1972). Zur medizinischen Bedeutung der Zecken in Österreich. Mitteilung der Abteilung Zoologie am Landesmuseum Joanneum Graz. 1, 2: 185-206

Skarphédinsson, S., Jensen, P. M., Kristiansen, K. (2005). Survey of tickborne infections in Denmark. Emerging Infectious Diseases. 11: 1055-1061

Skotarczak, B., Adamska, M., Sawczuk, M., Maciejewska, A., Wodecka, B., Rymaszewska, A. (2008). Coexistence of tick-borne pathogens in game animals and ticks in western Poland. Veterinarni Medicina. 53, 12: 668–675.

Sonnleitner, S. T., Fritz, J., Bednarska, M., Baumgartner, R., Simeoni, J., Zelger, R., Schennach, H., Lass-Flörl, C., Edelhofer, R., Pfister, K., Milhakov, A., Walder, G. (2014).

Risk assessment of transfusion-associated babesiosis in Tyrol: appraisal by seroepidemiology and polymerase chain reaction. Transfusion. 54: 1725-1732

Spahr, C., Knauf-Witzens, T., Vahlenkamp, T., Ulrich, R. G., Johne, R. (2017). Hepatitis E virus and related viruses in wild, domestic and zoo animals: A review. Zoonoses and Public Health. 65, 1: 11-29

Stanek, G. (2009). Büchse der Pandora: Krankheitserreger in *Ixodes ricinus*-Zecken in Europa [Pandora's Box: pathogens in *Ixodes ricinus* ticks in Central Europe]. Wiener klinische Wochenschrift. 121: 673-683

Stefanoff, P., Pfeffer, M., Hellenbrand, W., Rogalska, J., Rühle, F., Makówka, A., Michalik, J., Wodecka, B., Rymaszewska, A., Kiewra, D., Baumann-Popczyk, L., Dobler, G. (2013). Virus detection in questing ticks is not a sensitive indicator for risk assessment of tick-borne encephalitis in humans. Zoonoses and Public Health. 60, 3: 215-226

Süss, J. (2011). Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia-an overview. Ticks and Tick-borne Diseases. 2, 1: 2-15

Süss, J., Schrader, C., Falk, U., Wohanka, N. (2004). Tick-borne encephalitis (TBE) in Germany – epidemiological data, development of risk areas and virus prevalence in field-collected ticks and in ticks removed from humans. International Journal of Medical Microbiology. 69-79

Taylor, H. L., Latham, S. M., Woolhouse, M. E. L. (2001). Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.356: 983-989

Tiaoying, L., Jiamin, Q., Wen, Y., Craig, P.S., Xingwang, C., Ning, X., Ito, A., Giraudoux, P. (2005). Echinococcosis in Tibetan populations, Western Sichuan, province, China. Emerging Infectious Diseases. 11: 1866-1873

Tonteri, E., Jääskeläinen, A. E., Tikkakoski, T., Voutilainen, L., Niemimaa, J., Henttonen, H., Vaheri, A., Vapalahti, O. (2011**).** Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008–2009. Emerging Infectious Diseases. 17: 72-75

Tsaroucha, A. K., Polychronidis, A. C., Lyrantzopoulos, N., Pitiakoudis, M. S., Karayiannakis, A. J., Manolas, K. J., Simopoulos, C. E. (2005). Hydatid disease of the abdomen and other locations. World Journal of Surgery. 29: 1161–1165

Tylkowska, A., Pilarczyk, B., Pilarczyk, R., Zyśko, M., Tomza-Marciniak, A. (2019). Presence of tapeworms (Cestoda) in red fox (*Vulpes vulpes*) in north-western Poland, with particular emphasis on *Echinococcus multilocularis*. Journal of Veterinary Research 63: 71-78

Vannier, E., Krause, P. J. (2012). Human Babesiosis. The New England Journal of Medicine. 366: 2397-407

Vor, T., Kiffner, C., Hagedorn, P., Niedrig, M., Rühe, F. (2010). Tick burden on European roe deer (*Capreolus capreolus*). Experimental and Applied Acarology. 51: 405-417

Walder, G., Falkensammer, B., Heinz, F. X., Holzmann, H., Dierich, M. P., Würzner, R. (2008). Tick-borne encephalitis in the Tyrol (Austria): changes in incidence and endemicity 2000–2006. International Journal of Medical Microbiology. 298: 88-93

World Health Organisation (WHO) http://www.who.int/topics/zoonoses/en [abgefragt 05/2018]

Liste der Publikationen und Präsentation der Autorin

Originalbeitrag in Fachzeitschriften

Exp Appl Acarol (2017) 71:151–157 DOI 10.1007/s10493-017-0114-1
Tick abundance: a one year study on the impact of flood events along the banks of the river Danube, Austria

Beitrag für wissenschaftliche Veranstaltungen

Posterpräsentation (2014). 2ter Süddeutscher Zeckenkongress Schloss Hohenheim, Stuttgart/D. Der Vergleich der Zeckenfauna zweier benachbarter Augebiete mit unterschiedlichen Überflutungseigenschaften.

Populärwissenschaftlicher Beitrag

Jagd in Tirol (2016) Jahrgang 68: 46-47
Fuchsbandwurm: Erreger der gefährlichsten Parasitose in Mitteleuropa

Jagd in Tirol (2019) Jahrgang 71: 40-41
Vorsorgeuntersuchung Fuchsbandwurm: Ergebnisse aus den Jahren 2015 und 2016
Von allen 9 Bezirken in Tirol