Tick-Borne Infections Council of North Carolina, Inc.

NEWSLETTER 2017, Volume 4

Quote of the season: -

"In 1944, 39 percent of people living on the north shore of the lake tested positive for malaria. By the early 1950s, however, the disease had virtually disappeared from the state for reasons that are still not entirely understood, but improvements in mosquito control (especially the development of the pesticide DDT), drainage, housing, and nutrition probably all played a part."

– Excerpted from the entry by Peter McCandless in The South Carolina Encyclopedia, in the February 2017 SC Mosquito Association Newsletter.

Highlights…

Scroll down to see these features and more!

- **Where to find CDC case definitions and related information for Lyme disease**
- **TIC-NC activities**
- **More on lone star ticks as a vector of Rocky Mountain spotted fever**
- **New scientist coalition to work on tick-borne disease**
- **Ehrlichiosis is grossly under-reported, at least in NJ**
- **More on European species of the Lyme disease bacteria**

State Vector-Borne Disease Working Group 2017 Meeting Schedule

October 20, 2017 (Check with us before going to confirm date as they occasionally change.)

Location:
Office of the Chief Medical Examiner
4312 District Drive
Raleigh, NC 27607

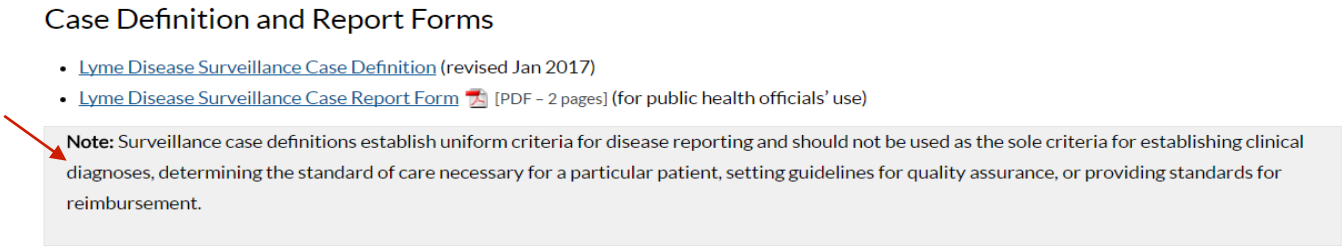
Photo ID required.

The Department of Health and Human Services, Division of Public Health, sends out yearly letters to Medical Providers on Lyme Disease and Rickettsial Diseases

This link connects you to our website publication page, where you will find current letters from NC Division of Public Health to medical providers, regarding Lyme disease and the Rickettsial diseases such as RMSF: http://tic-nc.org/publications/

Where To Find CDC case definitions and their statement that the surveillance case definitions are "not to be used as the sole criteria for establishing critical diagnosis"

Go to: www.cdc.gov/lyme/healthcare/index.html. (The links below in a clip of the website are not active.) Scroll down and find "Case Definition and Report Forms". See the grey box with "Note" containing the disclaimer.



Case Definition and Report Forms

- Lyme Disease Surveillance Case Definition (revised Jan 2017)
- Lyme Disease Surveillance Case Report Form [PDF – 2 pages] (for public health officials' use)

Note: Surveillance case definitions establish uniform criteria for disease reporting and should not be used as the sole criteria for establishing clinical diagnoses, determining the standard of care necessary for a particular patient, setting guidelines for quality assurance, or providing standards for reimbursement.

Accessed and copied 15 August 2017

Report from the Vectorborne Disease Work Group meeting – 08/2017 was cancelled.

State data on reportable tick-borne disease cases

Disease	Total cases by year of report 2014 Preliminary Confirmed + Probable (Confirmed/Probable/Suspected)*	2015 Final (Probable/Confirmed/Suspected)*	2016 Preliminary (Probable/Confirmed)
Lyme disease	170 (27/143/86)	192/38/46	277/33
Rickettsioses	496 (10/486/278)	454/5/130	475/6
Ehrlichioses	73 (11/62/31)	58/16/18	61/10
Anaplasmoses	12 (0/12/12)	15/4/3	14/1

This is the year of report, not year of illness onset.

Note: By the *former* CDC definition, six counties had confirmed cases of Lyme disease in two persons who had not traveled out of the county for 30 days after their tick exposure. **Therefore, these counties were endemic for Lyme disease by the former CDC definition: Wake, Guilford, Haywood, Alleghany, Buncombe, and Wilkes).** Counties with one case of locally acquired Lyme disease were: Cleveland (2008), Wilson (2009), Pitt (2009), Carteret (2009), Gates (2011), Perquimans (2011), Rowan (2013), Union (2013), Caldwell (2013), Franklin (2014), Stanley (2014), Duplin 2014.

TIC-NC Activities

During summer of 2017, TIC-NC participated in PORCH activites, distributing tick safety information and tick removers. Porch is a group of grass-roots volunteers in Chapel Hill, Carrboro, and Hillsborough who gather groceries from each others' porches on a monthly basis to feed the hungry in their areas. The numbers are kind of staggering, especially for North Carolina's second wealthiest county, Orange.

TIC-NC Talks and Materials Distributed

Brochures:

Botanical Gardens at Asheville
Park Veterinary Hospital, Durham
Vet hospital in Greensboro
Boehringer Ingelheim Animal Health, TN

HEALTHCARE MENTAL HEALTH OBESITY

CHATHAM HEALTH ALLIANCE
BUILDING COMMUNITY THROUGH COLLABORATION

TIC-NC had a booth 15 June 2017 at the Chatham County Health Alliance Fair. It was held at the new Agriculture Center – a lovely venue. Upwards of two dozen public and nonprofit health-related organizations participated so that we could all get to know one another and the services we offer. There was quite a lot of interest. We passed out a lot of our educational materials and had several requests for presentations.

Kim Brownley staffing our booth.

Lone star ticks competent vector of Rocky Mountain spotted fever in laboratory

Vector competence of *Amblyomma americanum* (Acari: Ixodidae) for *Rickettsia rickettsii*

Rickettsia rickettsii – the etiologic agent of Rocky Mountain spotted fever (RMSF) – is widely spread across the Americas. In the US, *Dermacentor* spp. ticks are identified as primary vectors of *R. rickettsii* and *Rhipicephalus sanguineus* s.l. has been implicated in transmission of this pathogen in several locations in the Southwest. Conversely, ticks of the genus *Amblyomma* are recognized vectors of RMSF in Central and South America, but not in the US. *A. americanum* is one of the most aggressive human-biting ticks in the US, whose geographical range overlaps with that of reported RMSF cases. Despite sporadic findings of *R. rickettsii* DNA in field-collected *A. americanum* and circumstantial association of this species with human RMSF cases, its vector competence for *R. rickettsii* has not been appropriately studied. Therefore, we assessed the ability of *A. americanum* to acquire and transmit two geographically distant isolates of *R. rickettsii*.

The Di-6 isolate of *R. rickettsii* used in this study originated in Virginia and the AZ-3 isolate originated in Arizona. Under laboratory conditions, *A. americanum* demonstrated vector competence for both isolates, although the efficiency of acquisition and transovarial transmission was higher for Di-6 than for AZ-3 isolate. Uninfected larvae acquired the pathogen from systemically infected guinea pigs, as well as while feeding side by side with *Rickettsia*-infected ticks on non-rickettsiemic hosts. Once acquired, *R. rickettsii* was successfully maintained through the tick molting process and transmitted to susceptible animals during subsequent feedings. Guinea pigs and dogs infested with infected *A. americanum* developed fever, scrotal edema and dermatitis or macular rash. *R. rickettsii* DNA was identified in animal blood, skin, and internal organs. The prevalence of infection within tick cohorts gradually increased due to side-by-side feeding of infected and uninfected individuals from 33–49% in freshly molted nymphs to 71–98% in engorged females. Moreover, *R. rickettsii* was transmitted transovarially by approximately 28% and 14% of females infected with Di-6 and AZ-3 isolates respectively.

Hence, *A. americanum* is capable of acquiring, maintaining and transmitting *R. rickettsii* isolates originating from two different geographical regions of the US, at least under laboratory conditions. Its role in ecology and epidemiology of RMSF in the US deserves further investigation. Levin ML et al. *Ticks and Tick-borne Diseases*, Available online 12 April 2017, In Press, Accepted Manuscript, http://www.sciencedirect.com/science/article/pii/S1877959X16303004

A review of studies on tick attachment times and disease transmission

Do Tick Attachment Times Vary between Different Tick-Pathogen Systems?

Improvements to risk assessments are needed to enhance our understanding of tick-borne disease epidemiology. We review tick vectors and duration of tick attachment required for pathogen transmission for the following pathogens/toxins and diseases: (1) *Anaplasma phagocytophilum* (anaplasmosis); (2) *Babesia microti* (babesiosis); (3) *Borrelia burgdorferi* (Lyme

disease); (4) Southern tick-associated rash illness; (5) *Borrelia hermsii* (tick-borne relapsing fever); (6) *Borrelia parkeri* (tick-borne relapsing fever); (7) *Borrelia turicatae* (tick-borne relapsing fever); (8) *Borrelia mayonii*; (9) *Borrelia miyamotoi*; (10) *Coxiella burnetii* (Query fever); (11) *Ehrlichia chaffeensis* (ehrlichiosis); (12) *Ehrlichia ewingii* (ehrlichiosis); (13) *Ehrlichia muris*; (14) *Francisella tularensis* (tularemia); (15) *Rickettsia* 364D; (16) *Rickettsia montanensis*; (17) *Rickettsia parkeri* (American boutonneuse fever, American tick bite fever); (18) *Rickettsia ricketsii* (Rocky Mountain spotted fever); (19) Colorado tick fever virus (Colorado tick fever); (20) Heartland virus; (21) Powassan virus (Powassan disease); (22) tick paralysis neurotoxin; and (23) Galactose-α-1,3-galactose (Mammalian Meat Allergy-alpha-gal syndrome).

Published studies for 12 of the 23 pathogens/diseases showed tick attachment times. Reported tick attachment times varied (<1 h to seven days) between pathogen/toxin type and tick vector. Not all studies were designed to detect the duration of attachment required for transmission. Knowledge of this important aspect of vector competence is lacking and impairs risk assessment for some tick-borne pathogens. Richards et al. *Environments* **2017**, *4*(2), 37; doi:10.3390/environments4020037

◘◘ National Section ◘◘

April 11, 2017 Announcement - For Distribution- new group of scientists interested in ticks and TBIs

Announcing the formation of a consortium of scientists titled, **"Tick Research to Eliminate Diseases: Scientist Coalition (TRED)."**TRED is a group of scientists, who joined together for synergy in promoting the need to address the ticks, the primary vector, as an answer to the environmental piece of this puzzle of Lyme and Tick-Borne Diseases. There has been a pittance of funding for this field, which holds such promise in its ability to solve the tick problem: reduce the tick population and tick ability to transmit pathogens.

TRED GOALS

1. Educate policy makers about the seriousness of tick-borne disease, and demonstrate the necessity and efficiency of addressing this serious problem at the source, the ticks.

2. Increase tick-borne disease research funding which specifically addresses the reduction of the tick population and/or blocking the ability of ticks to transmit all TBDs at federal, state and county levels.

3. Develop, test and implement research technologies to reduce incidence of tick-borne disease by reducing the risk of exposure to ticks and pathogens.

4. Coordinated implementation of these TBD prevention measures by federal, state, local governments and the public.

5. Representation by a Scientist in the field of Tick Research to the Federal Workgroup as described in Section 2062 of the 21st Century Cures Act.

The following is the list of scientists who are TRED members.

Tick Research to Eliminate Diseases: Scientist Coalition (TRED) Membership List

Apperson, Charles S., PhD
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Barbour, Alan G., MD
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Lab: Vector Borne Disease Diagnostics
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Professor & Director URI Center for Vector-Borne Disease, University of Rhode Island
http://www.tickencounter.org/
http://tickencounter.org/news/stuff_you_should_know_tick_image

Nieto, Nathan C, PhD
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Assistant Professor of Microbiology
Northern Arizona University, Department of Biological Sciences
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http://www.caryinstitute.org/science-program/our-scientists/dr-richard-s-ostfeld

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http://www.nature.com/articles/ncomms10507

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Wikel, Stephen, PhD
Professor emeritus, Frank H Netter MD, School of Medicine
Previous- Senior Associate Dean for Scholarship, Professor and Chairman, Department of Medical Sciences St. Vincent's Medical Center Endowed Chair Frank H. Netter, M.D., School of Medicine at Quinnipiac University and
Professor of Pathology, Center for Biodefense and Emerging Infectious Diseases
University of Texas Medical Branch
https://www.vectorbase.org/organisms/ixodes-scapularis/wikel
http://www.keystonesymposia.org/index.cfm?e=Web.Meeting.Flyer&MeetingID=1349
http://www.nature.com/articles/ncomms10507

CDC study suggests ehrlichiosis is grossly underreported (or misreported) or that many infections are asymptomatic, at least in New Jersey

Relative Risk for Ehrlichiosis and Lyme Disease in an Area Where Vectors for Both Are Sympatric, New Jersey, USA

The lone star tick, *Amblyomma americanum*, is a vector of *Ehrlichia chaffeensis* and *E. ewingii*, causal agents of human ehrlichiosis, and has demonstrated marked geographic expansion in recent years. *A. americanum* ticks often outnumber the vector of Lyme disease, *Ixodes scapularis*, where both ticks are sympatric, yet cases of Lyme disease far exceed ehrlichiosis cases. We quantified the risk for ehrlichiosis relative to Lyme disease by using relative tick encounter frequencies and infection rates for these 2 species in Monmouth County, New Jersey, USA. Our calculations predict ≥1 ehrlichiosis case for every 2 Lyme disease cases, >2 orders of magnitude higher than current case rates (e.g., 2 ehrlichiosis versus 439 Lyme disease cases in 2014). This result implies ehrlichiosis is grossly underreported (or misreported) or that many infections are asymptomatic. We recommend expansion of tickborne disease education in the Northeast United States to include human health risks posed by *A. americanum* ticks. Egizi et al. 2017. *Emerging Infectious Diseases*, *23*(6), 939-945.
https://wwwnc.cdc.gov/eid/article/23/6/16-0528_article.

The North Carolina veterinary school participated in this study.

Prevalence of Vector-Borne Pathogens in Southern California Dogs With Clinical and Laboratory Abnormalities Consistent With Immune-Mediated Disease

Studies investigating the prevalence of vector-borne pathogens in southern California dogs are limited. Occult infections might be misdiagnosed as idiopathic immune-mediated disease.

Hypothesis/Objectives To determine the prevalence of vector-borne pathogens in southern California dogs with compatible clinical findings using PCR and serologic panels and (2) to determine whether testing convalescent samples and repeating PCR on acute samples using the same and different gene targets enhance detection.

Methods Combined prospective and retrospective observational study. Forty-two acute and 27 convalescent samples were collected. Acute samples were prospectively tested for antibodies to *Rickettsia, Ehrlichia, Bartonella, Babesia, Borrelia, and Anaplasma species*. PCR targeting *Ehrlichia*, *Babesia*, *Anaplasma*, hemotropic *Mycoplasma,* and *Bartonella* species was also performed. Retrospectively, convalescent samples were tested for the same organisms using serology, and for *Ehrlichia*, *Babesia*, *Anaplasma*, and *Bartonella* species using PCR. Acute samples were retested using PCR targeting *Ehrlichia* and *Babesia* species.

Results Evidence of exposure to or infection with a vector-borne pathogen was detected in 33% (14/42) of dogs. *Ehrlichia* and *Babesia* species were most common; each was identified in 5 dogs. Convalescent serologic testing, repeating PCR, and using novel PCR gene targets increased detection by 30%.

Conclusions and Clinical Importance Repeated testing using serology and PCR enhances detection of infection by vector-borne pathogens in dogs with clinical signs of immune-mediated disease. Larger prevalence studies of emerging vector-borne pathogens in southern California dogs are warranted. Kidd et al. 30 May 2017, DOI: 10.1111/jvim.

Study on Children with Anaplasmosis

Clinical Presentation and Outcomes of Children With Human Granulocytic Anaplasmosis

Adults with the tick-borne disease human granulocytic anaplasmosis (HGA) have a spectrum of acute febrile illnesses that, if untreated, might be severe. Clinical presentation and outcomes of children with HGA have been poorly described.

A retrospective analysis was conducted to determine the frequency, presentation, and outcomes of pediatric patients with HGA between 1994 and 2015 in a region of Wisconsin in which HGA is highly endemic. Patients with related International Classification of Diseases Ninth and Tenth Revision (ICD-9 and ICD-10, respectively) codes or positive HGA laboratory test results were evaluated and classified as having had confirmed, probable, or suspected HGA on the basis of the Centers for Disease Control and Prevention (CDC) case definition. The Fisher's exact and Wilcoxon rank-sum tests were used in statistical comparisons.

Of 187 children identified with possible HGA, 17 (9%) had confirmed, 75 (40%) had probable, and 91

(49%) had suspected infections. The number of cases rose sharply in 2010 and has remained between 16 and 36 cases per year since that time. A minority of children with confirmed or probable infections had elevated liver transaminase levels (33%), leukopenia (24%), thrombocytopenia (17%), or anemia (8%); 6 (7%) of these children required hospitalization. Children with evidence of concurrent HGA and Lyme disease (27% of confirmed or probable cases) had a higher risk of hospitalization (odds ratio, 6.55 [95% confidence interval, 1.11–38.78]). None of these children had life-threatening disease or died.

Evidence suggests that the frequency of HGA in children is increasing. Although most children had mild disease, doxycycline remains the treatment of choice, because outcome data for children without treatment remains limited. Schotthoefer et al. J Pediatric Infect Dis Soc.https://doi.org/10.1093/jpids/pix029. May 2017

International & General Section

European red squirrels carry Lyme disease bacteria

Molecular detection of tick-borne pathogens *Borrelia afzelii*, *Borrelia miyamotoi* and *Anaplasma phagocytophilum* in Eurasian red squirrels (*Sciurus vulgaris*)

Eurasian red squirrels (*Sciurus vulgaris*) are common hosts of ixodid ticks and could thus carry tick-borne disease agents. The relative contribution of the red squirrel, a medium-sized rodent species, to the transmission dynamics of tick-borne pathogens in Europe yet remains unclear. We analysed spleen and liver samples from 45 dead squirrels collected in Flanders, Belgium, during tick activity season and detected the presence of *Borrelia burgdorferi* s.l. in the spleen of two squirrels (4.4%). One of the sequences could be identified as *Borrelia afzelii*. *Borrelia miyamotoi* was detected in the spleen of three squirrels (6.7%) and *Anaplasma phagocytophilum* in four spleen samples (8.9%). Both *A. phagocytophilum* ecotype I and II were found. We could not detect the presence of "*Candidatus* Neoehrlichia mikurensis" or tick-borne encephalitis virus in any of the squirrels.

Our results suggest that Eurasian red squirrels can host *B. afzelii*, as already proposed by previous studies, but we could not confirm the previous established association between squirrels and *B. burgdorferi* sensu stricto. Our results demonstrate the epidemiological importance of the red squirrel, particularly in (sub)urban areas, since they can harbour a similar community of tick-borne pathogens as do mice and voles and can act as hosts for *A. phagocytophilum* ecotype I, which has important implications for human health risk. Ruyts SC et al. *Eur J Wildl Res* (2017) 63: 43. doi:10.1007/s10344-017-1104-7

Areas of uncertainty in tick and disease control

Lyme disease ecology in a changing world: Consensus, uncertainty and critical gaps for improving control

Lyme disease is the most common tick-borne disease in temperate regions of North America, Europe and Asia, and the number of reported cases has increased in many regions as landscapes have been altered. Although there has been extensive work on the ecology and epidemiology of this disease in

both Europe and North America, substantial uncertainty exists about fundamental aspects that determine spatial and temporal variation in both disease risk and human incidence, which hamper effective and efficient prevention and control. Here we describe areas of consensus that can be built on, identify areas of uncertainty and outline research needed to fill these gaps to facilitate predictive models of disease risk and the development of novel disease control strategies.
Key areas of uncertainty include:

(i) the precise influence of deer abundance on tick abundance,
(ii) how tick populations are regulated,
(iii) assembly of host communities and tick-feeding patterns across different habitats,
(iv) reservoir competence of host species, and
(v) pathogenicity for humans of different genotypes of *Borrelia burgdorferi*.

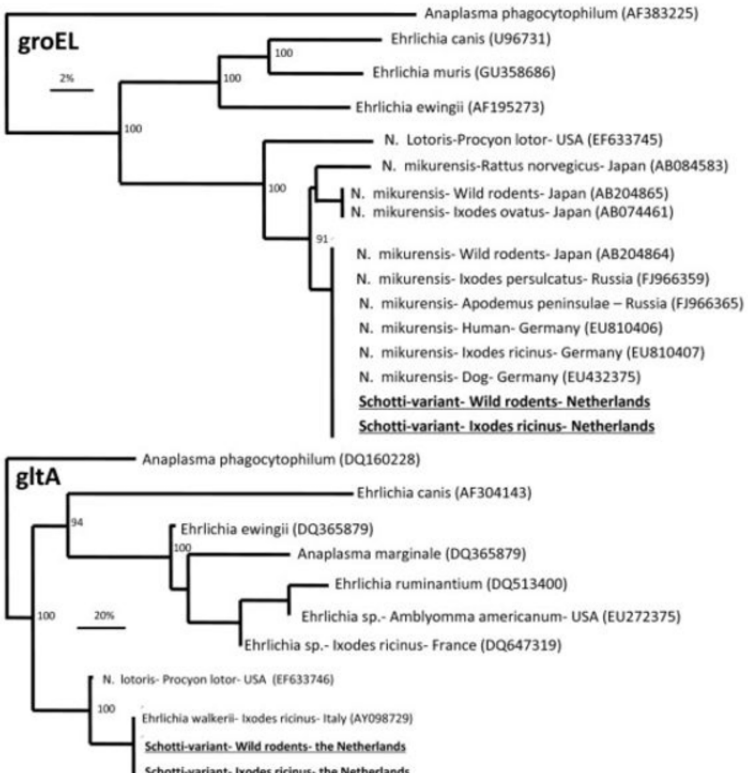
Filling these knowledge gaps will improve Lyme disease prevention and control and provide general insights into the drivers and dynamics of this emblematic multi-host–vector-borne zoonotic disease.
Kilpatrick et al. Philosophical Transactions of the Royal Society B: Biological Sciences, 372, Issue**:** 1722;2017

A European type of Ehrlichia (no common name) thought to be a pathogen in southern Norway and cause an erythema migrans-like rash

Candidatus Neoehrlichia mikurensis and Borrelia burgdorferi sensu lato detected in the blood of Norwegian patients with erythema migrans

The most common tick-borne human disease in Norway is Lyme borreliosis. Ticks in Norway also harbour less known disease-causing agents such as *Candidatus* Neoehrlichia mikurensis, *Borrelia miyamotoi* and *Rickettsia helvetica*. However, human infections caused by these pathogens have never been described in Norway. The main aims of the study were to evaluate the contribution of several tick-borne bacterial agents, other than *Borrelia burgdorferi* sensu lato, to zoonotic diseases in Norway and to determine their clinical pictures.

Blood samples from 70 symptomatic tick-bitten adults from the Agder counties in southern Norway were screened for seven tick-borne pathogens by using a commercial multiplex PCR-based method and by singleplex real-time PCR protocols. Most patients (65/70) presented with a rash clinically diagnosed as erythema migrans (EM). The most frequently detected pathogen DNA was from *Ca.* N. mikurensis and was found in the blood of 10% (7/70) of the patients. The *Ca.* N. mikurensis-infected patients presented with an EM-like rash as the only symptom. *B. burgdorferi* s.l. DNA was present in the blood of 4% (3/70) of the study participants. None had detectable *Anaplasma phagocytophilum*, *B.*



miyamotoi, *Rickettsia* typhus group or spotted fever group*, Francisella tularensis, Coxiella burnetii* or *Bartonella* spp. DNA in the blood.

The commercially available multiplex PCR bacteria flow chip system failed to identify half of the infected patients detected by corresponding real-time PCR protocols. The recovery of *Ca.* N. mikurensis DNA was higher in the pellet/plasma fraction of blood than from whole blood. To conclude, *Ca.* N. mikurensis appeared to be the etiological agent in patients with EM in a surprisingly large fraction of tick-bitten persons in the southern part of Norway. Quarsten et al. *Ticks and Tick-borne Diseases***,** https://doi.org/10.1016/j.ttbdis.2017.05.004

Editors note about *Ca*. N. mikurensis : from microbewiki.kenyon.edu/index.php/Neoehrlichia_mikurensis

Ca. N. mikurensis are gram negative cocci, generally 0.5-1.2µm in length. An obligate intracellular organism, it has been found in spleen tissue of rats infected with the bacterium. *Ca.* N. mikurensis is a pathogenic bacterium that has been found primarily in ticks and rodents in Europe and Asia. In 2007 three cases of confirmed human infection in and around Germany were reported, one of which was lethal. Based off of blood sample and 16S rRNA analyses from these cases, in 2010 it was determined that *Ca.* N. mikurensis was a novel human pathogen.

German study looking at Lyme disease bacteria in ticks finds a few infected larva and that most nymphs and adults are infected with *B. afzelii*

Lyme borreliae prevalence and genospecies distribution in ticks removed from humans

Lyme borreliosis (LB) is the most important human tick-borne disease, but *Borrelia* genospecies cause different clinical manifestations. Ticks of the genus *Ixodes* removed from humans between 2006 and 2012 were analysed for *Borrelia burgdorferi* sensu lato (sl) infections. The majority of ticks originated from the Greater Hanover region in Northern Germany. The engorgement status varied over the entire spectrum from unengorged (no evidence of started blood feeding) to fully engorged. In the present study, prevalence data for *B. burgdorferi* sl 2011 and 2012 were obtained by quantitative real-time PCR and compared to those from a former study including years 2006–2010 (Strube et al., 2011) to evaluate *B. burgdorferi* sl infections in ticks affecting humans over a 7-year period. In 2011, 34.2% (70/205) of adult ticks, 22.2% (94/423) of nymphs, 8.3% of larvae (1/12) as well 3 of 6 not differentiated ticks were *Borrelia* positive. In 2012, 31.8% (41/129) of adult ticks, 20.4% of nymphs (69/337) as well as 1 of 4 of the not differentiated ticks were determined positive.

Total *Borrelia* infection rates decreased significantly from 23.1% in 2006 to 17.1% in 2010, followed by a significant increase to 26.0% in 2011 and 23.4% in 2012. Furthermore, *B. burgdorferi* sl genospecies distribution in 2006–2012 was determined in the present study by applying Reverse Line Blot technique. *Borrelia* genospecies differentiation was successful in 641 (67.3%) out of 953 positive tick samples.

The most frequently occurring genospecies was *B. afzelii* (40.5% of infected ticks), followed by *B. garinii*/*B. bavariensis* (12.4%). Amongst the 641 ticks analysed for their genospecies, 74 (11.5%) carried more than one genospecies, of which 69 (10.7%) were double-infected and five (0.8%) were triple-infected. Comparison of genospecies distribution in ticks removed from humans with those from questing ticks flagged in the same geographical area revealed that ticks removed from humans were significantly more frequently infected with *B. afzelii* ($p = 0.0004$), but significantly less infected with *B. burgdorferi* sensu stricto ($p = 0.0001$). *Ticks and Tick-borne Diseases***,** Waindock et al. doi.org/10.1016/j.ttbdis.2017.05.003.

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Tick-Borne Infections Council of North Carolina is a non-profit 501(c)3 organization formed to improve the recognition, treatment, control, and understanding of tick-borne diseases in North Carolina. We are all-volunteer and appreciate donations.

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