January 2013

Hypersensitivity Reactions To Ixodes Scapularis Bites And Protection Against Tick-Borne Infection

Gregory Kimball Nielsen
Yale University, gregory.nielsen@yale.edu

Hypersensitivity reactions to *Ixodes scapularis* bites and protection against tick-borne infection

Gregory Kimball Nielsen

A Thesis Presented to the Faculty of the Yale School of Public Health
Yale University
In Candidacy for the Degree of Master of Public Health
2013

First Thesis Reader: Peter J. Krause, M.D.

Second Thesis Reader: Sukanya Narasimhan, Ph.D.

Abstract

Background: Salivary proteins from insect bites result in a wide array of complex immune interactions within a bitten host. In the case of the deer tick, *Ixodes scapularis,* previous research has demonstrated that tick-induced hypersensitivity reactions may interfere with the transmission of Lyme disease. There are no prospective studies in humans regarding the spectrum of hypersensitivity reactions that occur with *I. scapularis* bites.

Methods: We analyzed data obtained from a prospective enrollment of the first 102 individuals who reported tick bite to a medical practice in Mansfield, Connecticut from 2005-2008. Clinical responses were recorded and subject based diaries were utilized to classify and analyze whether certain reactions reduced tick-borne pathogen transmission.

Results: No subjects developed serious clinical manifestations or systemic reactions. The most common localized reactions were local erythema (88%), swelling (64%), itch (48%), and a delayed type hypersensitivity reaction (27%). None of these responses were associated with the presence or absence of a previous episode of Lyme disease.

Conclusion: Hypersensitivity reactions to *I. scapularis* bites generally are mild. Although they may help to prevent tick-borne infection, we did not observe an association between tick-bite reaction and the presence or absence of Lyme disease. An expanded and modified surveillance study is needed to determine if there is an association between hypersensitivity reactions and the development of tick-borne infection.

Acknowledgments

I would like to thank the dedicated team of researchers and health professionals that collected the data for this research. Dr. Sukanya Narasimhan for her aid and input as a thesis reader. Dr. Maria Diuk-Wasser, Dr. Michael Capello, and the Capello Laboratory for their guidance over the summer. Lastly, Dr. Peter Krause for his mentorship throughout my Yale School of Public Health experience.

Table of Contents

Introduction

When a tick feeds on a mammalian host, salivary proteins are introduced into the skin that can potentially cause a cutaneous hypersensitivity reaction (1). The development of major health risks from these reactions are infrequently diagnosed, but repeated exposure to tick bites can induce an itch response (2). Such a response may serve as a method of tick detection, allowing subsequent removal of the tick prior to pathogen transmission. In the case of Lyme disease, the causative pathogen *Borrelia burgdorferi* is transmitted from the tick to the host 36 to 72 hours into the blood meal. Ticks inject salivary kinases that break down bradykinin, an itch-inducing host protein (3). This serves as a protective method for a feeding tick, allowing it to remain undetected and to complete a blood meal. After multiple bites, a host can develop an immunologic response that destroys these kinases, allowing the itch reaction and potentially helping to prevent transmission of certain tick-borne pathogens. A previous study has shown that individuals who experience repeated tick bite associated itch have a lower risk of acquiring Lyme disease (4). Aside from immediate cutaneous reactions, delayed type hypersensitivity may also be protective against tick-borne pathogen transmission. Immune cells infiltrate the bite site after 24 hours resulting in swelling and induration (5). This could potentially interfere with tick feeding and pathogen transmission and survival at the bite site in the host (8,9). With the emergence of Lyme disease and other *Ixodes scapularis* tick-borne diseases like anaplasmosis and babesiosis, understanding human-tick immune interactions may allow the creation of a salivary protein based vaccine. Such a vaccine could potentially protect a person from multiple tick-borne diseases.

The limited number of population-based tick bite studies prompted us to carry out this research project so that we could classify the scope of human tick bite reactions. We also sought to determine whether delayed type hypersensitivity reactions are common, since the swelling and immune cell infiltration associated with this response could interfere with pathogen transmission or kill pathogens at the bite site (10,11). To our knowledge, the frequency of tick bite delayed type hypersensitivity reactions for humans has yet to be evaluated. Finally, we sought to determine whether increased reactions are induced by multiple tick bites and whether they protect against tick-borne illnesses. We hypothesized that tick bites primarily induce localized cutaneous reactions and that systemic and clinically severe reactions are minimal. We also hypothesized that subjects who experience itch and other cutaneous hypersensitivity reactions are less likely to experience tick-borne infection.

Background

There are many pathogens throughout the world that are transmitted through tick bites (12). Complex immune reactions develop from these infections but there are also fascinating immune interactions that develop between ticks and their hosts (13). Bites from certain species of tick can result in serious health complications like tick paralysis from *I. holocyclus* in Australia (4). The most common immune reactions to tick bites are cutaneous hypersensitivity reactions, primarily immediate hypersensitivity and delayed type hypersensitivity (4,14-17). These reactions are of particular interest for two reasons. Firstly, both immediate and delayed type hypersensitivity have been shown to have a protective effect against tick-borne pathogen transmission (8-10,15). Secondly, both of these reactions can potentially be induced by multiple exposures to tick salivary proteins. Cutaneous hypersensitivity reactions may allow scientists to produce a tick

salivary protein based vaccine that could protect an individual from multiple tick-borne infections.

Immediate Hypersensitivity

Immediate hypersensitivity responses are the result of a rapid production of IgE, accumulation of eosinophils, and the degranulation of mast cells and basophils (18). Swelling, erythema, and itch typically develop from immediate hypersensitivity reactions. Although uncommon, anaphylaxis has been reported following tick bite (2,19,20). Tick based immediate hypersensitivity reactions can be induced in many different species of mammals suggesting that this reaction is common throughout this taxonomic lineage (14, 18, 5-7, 21, 22). Within minutes of a tick bite, immediate hypersensitivity rapidly develops and then diminishes within an hour (5). Tissue samples from BALB/c mice bitten multiple times has shown sustained levels of eosinophils and mast cells regardless of bite frequency; however more mast cells will undergo degranulation with each successive bite (5). The development of these cutaneous hypersensitivity reactions is a response to tick salivary protein.

Various tick salivary proteins have been identified to have antigenic properties. Researchers isolated a 84 kDa salivary protein that induced hypersensitivity reactions in rabbits that were previously exposed to ticks (16). Furthermore, people previously bitten by ticks produce antibodies against the tick salivary protein calreticulin (23). Salivary proteins appear to play a clear role in the development of immediate hypersensitivity reactions. The development of delayed type hypersensitivity reactions to tick bites is not as well understood.

Delayed Type Hypersensitivity

A delayed type hypersensitivity reaction consists of the accumulation of immune cells, predominantly T cells, after a 24-hour period following the causative stimulus. The presence of tick induced delayed type hypersensitivity reactions may or may not develop depending on the species of mammal. Dogs have not been observed to exhibit these reactions while they are common within guinea pigs. Presence of a response also varies by breed, as in the case of mice and cattle (10, 21,17,11). Cellular infiltration of monocytes, lymphocytes, and neutrophils accumulate after 24 hours and eventually reach a peak 72 to 92 hours into a tick feeding. This accumulation of cells results in swelling and induration at the bite site (5). As with immediate hypersensitivity reactions, the response becomes more pronounced with successive tick bites (5).

Symptoms of tick-induced cutaneous delayed type hypersensitivity reactions have not been well studied in humans. The TST skin test used to detect tuberculosis induces a cutaneous delayed hypersensitivity reaction in people who have been exposed to tuberculosis and has many similarities to tick induced delayed type hypersensitivity. The TST skin test is a sub-dermal injection similar to that used to induce delayed hypersensitivity reactions in laboratory animals using tick salivary proteins (24). The size of swelling and induration that develop at the injection site are used to define a positive TST. Optimal levels of swelling occur 48 to 72 hours after exposure, similar to tick induced delayed type hypersensitivity reactions in animals (24,25). Moreover both TST testing and tick induced delayed type hypersensitivity in animal models are associated with elevated levels of IFN- γ and TNF- α (6,7,26).

There is limited published information regarding tick-induced delayed type hypersensitivity in humans, however, clinicians in France confirmed a delayed type

hypersensitivity in a patient bitten by an *I. ricinus* tick (1). The patient experienced both swelling and induration at the bite site and had a history of tick bites suggesting that the reaction can be induced in humans after multiple tick bites. Another study demonstrated that skin biopsies obtained from a human subject bitten by multiple ticks exhibited similar immune cell infiltration found in mice (8).

Considering the similarities between the TST skin test, animal reactions, and the limited human studies, it is reasonable to propose that the onset of swelling and induration from a tick bite lasting at least 24 hours after the bite can be classified as a tick-induced delayed type hypersensitivity reaction. Such criteria will provide a non-invasive measure of the presence of delayed type hypersensitivity reactions and help estimate their prevalence in people exposed to *I.scapularis* bites.

Hypersensitivity and protection from tick-borne illness

As stated previously, itch may allow a host to detect a tick and remove it before *B. burgdorferi* can be transmitted. One study found that individuals who experience multiple occurrences of itch have a lower risk of acquiring Lyme disease (4). Other cutaneous reactions may also interfere with tick feeding and disease transmission. Following an initial tick bite, blood vessels dilate and provide the tick better access for its blood meal. Additional blood meals may result in decreased vascular size, as well as infiltration of neutrophils, lymphocytes, and eosinophils (8). When guinea pigs were bitten by *B. burgdorferi* infected ticks, the engorgement weights and the duration of attachment were less in ticks that fed on animals that had previously experienced tick bite than on animals that had not previously been bitten. The animals were also less likely to acquire Lyme disease. Transmission of the pathogen occurs 48 to 72 hours

after a tick bite and most of the ticks detached within 24 hours from the animals that previously had been bitten (27). Similar testing of BALB/c mice confirmed smaller engorgement weights and less *B. burgdorferi transmission* for mice with prior tick exposure (15). Tissue samples collected within 24 hours illustrated that animals that rejected ticks had increased levels of degranulated mast cells and an increased number of eosinophils and basophils, indicating an immediate hypersensitivity response (9). The role of delayed type hypersensitivity reactions in preventing tick feeding is not as well understood. It has been proposed that the increased infiltration of immune cells later in the tick exposure can provide an enhanced response against tick-borne pathogens (10).

Methods

Study design

Study subjects were enrolled between the months of April and October from 2005 to 2008. Data was collected for patients who came to the Mansfield Family Practice in Mansfield, Connecticut who were bitten by a tick within the previous 48-hour period. Patients completed a standardized questionnaire to determine tick bite history, previous bite reactions, and existing allergies. Patients were also asked if they had ever been diagnosed with a previous tick-borne infection, including Lyme disease, babesiosis, human granulocytic anaplasmosis, and human ehrlichiosis. Patients were examined by a physician, physician assistant, or nurse practitioner and they recorded any reaction that was present.

After the visit, patients were provided with a standardized tick-bite reaction diary. The diary consisted of questions that allowed subjects to monitor tick bite manifestations, including itch, redness, hardness, swelling, and pain. Diaries were recorded until signs and symptoms resolved. A blood sample was collected during their

initial visit and 4-6 weeks later, at which time the diary was also collected. Written informed consent was obtained from all subjects in accordance with the Institutional Review Boards at the Connecticut Children's Medical Center, Hartford, Connecticut and the University of Connecticut Health Center, Farmington, Connecticut.

Delayed type hypersensitivity

Patients who experienced both swelling and a hard lump at the site of the tick bite that lasted for more than 24 hours were classified as having had a delayed type hypersensitivity reaction. Redness was not considered for classification. Patients that exhibited swelling and a hard lump, but had reactions that lasted less than 24 hours were excluded from the classification.

Statistical analysis

Statistical analyses were performed with SAS version 9.2 (SAS Institute Inc. Cary, NC,USA). Comparisons for tick bite history and present reactions utilized a one-way analysis of variance (ANOVA). Comparing tick bite reactions between those who experienced itch and those who did not used an unpaired t-test for evaluation. Simple logistic regression was used to evaluate data where odds ratios (OR) and 95% confidence intervals (CI) were calculated. Missing values were excluded from the analysis and statistically significant data was defined as a P-value of less than 0.05.

Results

During the 2005 to 2008 study period, 102 individuals were enrolled in the study. There were two patients who experienced two tick bites and who were enrolled twice. One patient was bitten three times and enrolled three times. None of the subjects that were

repeatedly enrolled in the study experienced an increase in severity of their tick bite reactions.

The first step of analysis was to summarize demographic information, the frequency of tick bite reactions, and prior tick-borne illnesses within the study population (Table 1). There were no serious clinical manifestations or systemic reactions (such as fever headache, or fatigue) that developed in any of the patients. Erythema was the most common tick bite reaction appearing in almost all (88%) of the study population. None of the reports of erythema at the site of the tick bite were 5 cm or greater in diameter, the minimum size required to diagnose erythema migrans. Other reactions in decreasing order of frequency were: swelling, itch, hard lump, delayed type hypersensitivity, and pain. A past incidence of Lyme disease occurred in about a third (37%) of the study subjects and only two subjects were previously diagnosed with ehrlichiosis. No other tick-borne illnesses were reported.

Tick bite reactions were further classified in regard to frequency of tick bites over the previous five year period (Table 2). Itch was the only reaction that increased significantly with increasing number of tick bites and appeared in most (82%) individuals who had been bitten more than five times. Duration of a tick bite reaction was not influenced by tick bite frequency. Further analysis was performed regarding itch and tick bite frequency in the previous twelve months (Table 3). Subjects who had been bitten three or more times within the previous year had greater probability of developing an itch reaction (OR 6.5 CI 1.46-28.80 $P<0.01$). Within the five-year period, subjects bitten more than five times also had greater chance of developing an itch reaction (OR 16.0 CI 2.65-96.47). Subjects bitten one to five times in the previous five years were not likely to develop itch when compared to the control group. Erythema, swelling, hard lump, and

delayed hypersensitivity reactions were more common in subjects who experienced itch. Both erythema and swelling appeared for all of the subjects in the itch group. Delayed type hypersensitivity was classified in a little more than half (63%) of those who experienced itch and only 8% of individuals who did not.

Further analysis was performed to determine odds ratios for the reactions that were significantly related to itch (Table 4). All of the reactions had statistically significant odds ratios that indicated positive association with the development of an itch reaction. Furthermore, those who experienced itch in the past had greater odds of experiencing itch from a current tick bite (OR 11.94 CI 3.25-43.89 P <0.01).

Delayed type hypersensitivity (OR 18.86 CI 3.37-105.49 P <0.01) was compared in subjects who developed swelling reactions with no hard lump and subjects who developed a hard lump and no swelling (Table 5). Neither the subjects experiencing swelling alone or the hard lump alone were more likely than controls to develop an itch response. It should be acknowledged that the swelling only group had a p-value of 0.06 and might have been significant with a larger sample size. Delayed type hypersensitivity and tick bite history were analyzed in a similar fashion as itch reaction (Table 3) and subjects who were bitten more than five times had higher odds of having a delayed type hypersensitivity reaction (OR 11.67 CI 1.14-119.55 P=0.04) (data not shown).

Due to the low frequency of other diseases within the study population, only Lyme disease was analyzed to determine if certain tick bite reactions might have a protective effect against tick-borne illness. Tick-bite frequency and its relation to past diagnosis of Lyme disease were analyzed for the prior twelve month and five year periods (Table 6). Individuals bitten two times (OR 12.5 CI 1.19-130.62 P= 0.04) and more than two times (OR 17.50 CI 1.76-174.43 P=0.01) in the previous twelve month

period had greater odds of previously having had Lyme disease. Over a five year period individuals bitten more than five times (OR 52.78 2.52-1104.6 P= 0.01) had greater odds of previously having had Lyme disease diagnosis.

Odds ratios were calculated to determine whether or not subjects with particular reactions were less likely to have had a past Lyme disease diagnosis (Table 7). Although the development of erythema, pain, and hard lump had odds ratios indicating a protective effect, and all of the reactions had confidence intervals indicating the potential for protection, none of these associations were statistically significant. Further analysis indicated that there was no association with noticing a tick and the development of itch or delayed type hypersensitivity (data not shown).

Conclusions

The results of our study suggest that most individuals who react to an *I. scapularis* tick bite develop a mild local dermatologic reaction. We did not observe serious reactions like anaphylaxis among the 102 people who experienced tick bite, supporting the hypothesis that this type of reaction is uncommon for tick bites. In contrast, anaphylaxis resulting from the sting of bees and wasps occurs in 1.2-3.5% of the population (28). We found that hypersensitivity reactions commonly occur after a tick bite and include erythema, itch, swelling, and induration. Although these reactions have the potential to reduce the risk of Lyme disease infection, our data did not provide definitive conclusions in this regard.

Tick bite reactions may protect against tick-borne pathogen infection in several ways. An itch reaction to tick bite can allow the detection of a feeding tick and removal before a pathogen is transmitted. Swelling at the bite site can prevent the normal feeding process and prevent pathogen transmission. Movement of inflammatory cells

such as neutrophils to the bite site may kill pathogens deposited at the site. Previous research indicates that individuals who have persistent itch reactions are less likely to be susceptible to *B. burgdorferi* infection (4). In the same study, a single incidence of itch conveyed no protection towards Lyme disease transmission. Taken together, this data and that from our current study suggest that development of itch either is not always an effective method of tick detection and associated prevention of Lyme disease, or that some itch reactions occur too late to prevent *B. burgdorferi* transmission, or that other reactions associated with itch may be responsible for prevention of Lyme disease.

We corroborated that the prevalence of itch reaction is related to the number of times an individual is bitten by tick. The development of this reaction may vary on an individual basis, but people who are bitten more than five times are very likely to develop an itch response. Multiple tick bites result in elevated production of IL-4 that helps differentiate naïve T cells into Th2 cells that then can initiate immediate hypersensitivity (6). Tick saliva has been shown to suppress production of IL-4, which may explain why itch does not occur after an initial tick bite (29). Our data also showed that delayed type hypersensitivity reactions can be induced by *I. scapualris* bites, however fewer individuals develop this reaction than itch. Delayed type hypersensitivity reactions only occur within a subset of the population. This observation is consistent with the fact that certain breeds of cattle are able to develop delayed type hypersensitivity reactions from tick bites while other breeds are incapable of producing this type of reaction regardless of exposure (11,17).

Our study was subject to several limitations. Firstly, in regard to determining the prevalence of severe reactions, it is worth considering that individuals with serious

reaction will likely go directly to the hospital instead of a primary care clinic where we carried out our study. Secondly, while our data provides an initial analysis of the range of reactions that occur from tick bites; an expanded surveillance system would improve our understanding of the array of reactions by increasing sample size. Prior clinical reports indicate that the diagnoses for clinically serious reactions such as anaphylaxis are relatively uncommon (2,19,20). Mortality from *I. scapularis* bites has yet to be documented. Throughout the United States an average of 56 people die from bee and wasp stings every year (30). The fact that *I. scapularis* ticks are concentrated within specific regions of the United States and the low occurrence of tick bite anaphylaxis both contribute to the lower rate of serious reactions compared to that of bee stings and thus are harder to detect. Future studies to evaluate the incidence of severe and systemic reactions from tick bites should expand surveillance systems to include hospitals, as well as a network of primary care facilities. A final limitation of our study was that subjects were susceptible to recall bias because of the retrospective nature of the tick bite diaries. Questions regarding the number of past tick bites, reactions experienced from these bites, and the development of Lyme disease could have led to both under and over reporting. A potential useful approach to address this problem would be to develop a study cohort living in a highly endemic area for Lyme disease with no prior tick bite history. Several years of data collection would provide a more accurate assessment of the number of tick bites that an individual experiences, the reactions that accompany each bite, and possible association with tick-borne illness. Such research would prove valuable information pertaining to our research questions.

Ours study provides an initial assessment of population responses to *I. scapularis* bites. With the emergence of Lyme disease and other tick-borne pathogens,

understanding reactions from individuals bitten by uninfected ticks will allow physicians to better diagnose individuals with disease manifestations. Whether certain tick-bite reactions protect individuals from pathogen transmission requires further evaluation. The potential benefits of establishing such an association warrant further research.

References

1. Beaudouin E, Kanny G, Guerin B, Guerin L, Plenat F, Moneret-Vautrin DA. Unusual manifestations of hypersensitivity after a tick bite: report of two cases. Ann Allergy Asthma Immunol. 1997 Jul;79(1):43-6.
2. Dantas-Torres F. Anaphylactic shock caused by tick bites. J Investig Allergol Clin Immunol. 2008;18(3):231.
3. Ribeiro JM, Spielman A. Ixodes dammini: salivary anaphylatoxin inactivating activity. Exp Parasitol. 1986 Oct;62(2):292-7.
4. Burke G, Wikel SK, Spielman A, Telford SR, McKay K, Krause PJ; Tick-borne Infection Study Group. Hypersensitivity to ticks and Lyme disease risk. Emerg Infect Dis. 2005 Jan;11(1):36-41.
5. Mbow ML, Christe M, Rutti B, Brossard M. Absence of acquired resistance to nymphal Ixodes ricinus ticks in BALB/c mice developing cutaneous reactions. J Parasitol. 1994 Feb;80(1):81-7.
6. Mbow ML, Rutti B, Brossard M. IFN-gamma IL-2, and IL-4 mRNA expression in the skin and draining lymph nodes of BALB/c mice repeatedly infested with nymphal Ixodes ricinus ticks. Cell Immunol. 1994 Jun;156(1):254-61.
7. Mbow ML, Rutti B, Brossard M. Infiltration of CD4+ CD8+ T cells, and expression of ICAM-1, Ia antigens, IL-1 alpha and TNF-alpha in the skin lesion of BALB/c mice undergoing repeated infestations with nymphal Ixodes ricinus ticks. Immunology. 1994 Aug;82(4):596-602.
8. Krause PJ, Grant-Kels JM, Tahan SR, Dardick KR, Alarcon-Chaidez F, Bouchard K, Visini C, Deriso C, Foppa IM, Wikel S. Dermatologic changes induced by repeated Ixodes scapularis bites and implications for prevention of tick-borne

infection. Vector Borne Zoonotic Dis. 2009 Dec;9(6):603-10. doi: 10.1089/vbz.2008.0091.

9. Narasimhan S, Deponte K, Marcantonio N, Liang X, Royce TE, Nelson KF, Booth CJ, Koski B, Anderson JF, Kantor F, Fikrig E. Immunity against Ixodes scapularis salivary proteins expressed within 24 hours of attachment thwarts tick feeding and impairs Borrelia transmission. PLoS One. 2007 May 16;2(5):e451.
10. Ferreira BR, Szabó MJ, Cavassani KA, Bechara GH, Silva JS. Antigens from *Rhipicephalus sanguineus* ticks elicit potent cell-mediated immune responses in resistant but not in susceptible animals. Vet Parasitol. 2003 Jul 10;115(1):35-48.
11. Prudencio CR, Rezende Rodrigues AA, Cardoso R, de Souza GR, Szabó MP, Goulart LR. Cutaneous hypersensitivity test to evaluate phage display anti-tick borne vaccine antigen candidates. Exp Parasitol. 2011 Dec;129(4):388-92. doi: 10.1016/j.exppara.2011.08.017.
12. Elston DM. Tick bites and skin rashes. Curr Opin Infect Dis. 2010 Apr;23(2):132-8.
13. Castelli E, Caputo V, Morello V, Tomasino RM. Local reactions to tick bites. Am J Dermatopathol. 2008 Jun;30(3):241-8.
14. Gauci M, Loh RK, Stone BF, Thong YH. Allergic reactions to the Australian paralysis tick, Ixodes holocyclus: diagnostic evaluation by skin test and radioimmunoassay. Clin Exp Allergy. 1989 May;19(3):279-83.
15. Wikel SK, Ramachandra RN, Bergman DK, Burkot TR, Piesman J. Infestation with pathogen-free nymphs of the tick Ixodes scapularis induces host resistance to transmission of Borrelia burgdorferi by ticks. Infect Immun. 1997 Jan;65(1):335-8.
16. Mulenga A, Sugimoto C, Ohashi K, Onuma M. Characterization of an 84 kDa protein inducing an immediate hypersensitivity reaction in rabbits sensitized to Haemaphysalis longicornis ticks. Biochim Biophys Acta. 2000 Jun 15;1501(2-3):219-26.
17. Bechara GH, Morelli Júnior J, Szabó MP. Skin test and tick immune status in susceptible and resistant cattle in Brazil. Ann N Y Acad Sci. 2000;916:570-5.
18. Christe M, Rutti B, Brossard M. Susceptibility of BALB/c mice to nymphs and larvae of Ixodes ricinus after modulation of IgE production with anti-interleukin-4

or anti-interferon-gamma monoclonal antibodies. Parasitol Res. 1998 May;84(5):388-93.

19. Van Wye JE, Hsu YP, Lane RS, Terr AI, Moss RB. IgE antibodies in tick bite-induced anaphylaxis. J Allergy Clin Immunol. 1991 Dec;88(6):968-70.
20. Acero S, Blanco R, Bartolomé B. Anaphylaxis due to a tick bite. Allergy. 2003 Aug;58(8):824-5.
21. Schoeler GB, Manweiler SA, Wikel SK. Ixodes scapularis: effects of repeated infestations with pathogen-free nymphs on macrophage and T lymphocyte cytokine responses of BALB/c and C3H/Hen mice. Exp Parasitol. 1999 Aug;92(4):239-48.
22. Vychodilova L, Matiasovic J, Bobrova O, Futas J, Klumplerova M, Stejskalova K, Cvanova M, Janova E, Osickova J, Vyskocil M, Sedlinska M, Dusek L, Marti E, Horin P. Immunogenomic analysis of insect bite hypersensitivity in a model horse population. Vet Immunol Immunopathol. 2013 Apr 15;152(3-4):260-8. doi: 10.1016/j.vetimm.2012.12.013.
23. Alarcon-Chaidez F, Ryan R, Wikel S, Dardick K, Lawler C, Foppa IM, Tomas P, Cushman A, Hsieh A, Spielman A, Bouchard KR, Dias F, Aslanzadeh J, Krause PJ. Clin Vaccine Immunol. Confirmation of tick bite by detection of antibody to Ixodes calreticulin salivary protein. Alarcon-Chaidez F 2006 Nov;13(11):1217-22. Epub 2006 Aug 23.
24. American Thoracic Society. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med. 2000 Apr;161(4 Pt 1):1376-95.
25. Girardin P, Brossard M. Effects of cyclosporin A on humoral immunity to ticks and on cutaneous immediate and delayed hypersensitivity reactions to Ixodes ricinus L. salivary-gland antigens in re-infested rabbits. Parasitol Res. 1989;75(8):657-62.

26. Subash Babu, Sajid Q. Bhat, N. Pavan Kumar, V. Kumaraswami, and Thomas B. Nutman. Regulatory T Cells Modulate Th17 Responses in Tuberculin skin test positive (TST+) individuals. J Infect Dis. 2010 January 1; 201(1): 20–31.
27. Nazario S, Das S, de Silva A M, Deponte K, Marcantonio N, Anderson J F, Fish D, Fikrig E, Kantor F S. Prevention of Borrelia burgdorferi transmission in guinea pigs by tick immunity.The American journal of tropical medicine and hygiene (impact factor: 2.59). 07/1998; 58(6):780-5.
28. Przybilla B, Ruëff F: Insect stings: clinical features and management. Dtsch Arztebl Int 2012; 109(13): 238–48. DOI: 10.3238/arztebl.2012.0238
29. Mejri N, Rutti B, Brossard M. Immunosuppressive effects of ixodes ricinus tick saliva or salivary gland extracts on innate and acquired immune response of BALB/c mice. Parasitol Res. 2002 Mar;88(3):192-7.
30. Centers for Disease Control and Prevention, National Center for Health Statistics. Compressed Mortality File""1999-2010 on CDC WONDER Online Database, released January 2013. Data are compiled from Compressed Mortality File 1999-2010"

Appendix

Table 1. Description of patient population as well as tick bite related illnesses and reactions.

Characteristic	Total (N=102)
Age	
Mean	53
Median	57
Range	5-77
Number of years in Connecticut	
Mean	36.63
Median	37.50
Range	2-77
Male sex – no. (%)	68 (67.3)
History of tick-borne illnesses	
Lyme disease	21 (36.8)
Babesiosis	0 (0)
Ehrlichiosis	2 (4.1)
Reactions to current tick bite	
Redness – no. (%)	59 (88.1)
Swelling – no. (%)	37 (63.8)
Induration – no. (%)	25 (44.6)
Itch – no. (%)	28 (47.5)
Delayed Type Hypersensitivity- no. (%)	14 (26.9)
Pain – no. (%)	10 (19.6)
No Reaction- no. (%)	8 (11.4)
Reactions to past tick bite	
Redness – no. (%)	40 (62.5)
Swelling – no. (%)	28 (49.1)
Induration – no. (%)	19 (35.2)
Itch – no. (%)	26 (47.3)
Pain – no. (%)	11 (22.9)

Numbers may not sum to total due to missing data.

Table 2. Skin bite reactions among people who reported varying exposure to tick bite over the last five years.

Characteristic	None (N=14)	1-5 tick bites (N=31)	>5 tick bites (N=21)	P value*
Erythema	14 (100.0)	24 (80.0)	18 (94.7)	0.09
Swelling	5 (41.7)	16 (61.5)	14 (87.5)	0.51
Induration	6 (46.2)	9 (36.0)	9 (60.0)	0.35
Itch	**3 (25.0)**	**8 (32.0)**	**16 (84.2)**	**<0.01**
Delayed Type Hypersensitivity	4 (50.0)	8 (34.7)	1 (7.7)	0.09
Pain	4 (33.3)	5 (20.8)	0 (0.0)	0.13
No Reaction	0 (0.0)	5 (21.7)	1 (10)	0.13
Duration of reaction				
One hour or less	1 (7.1)	1 (3.2)	1 (5.3)	0.32
Between 1 and 24 hours	7 (50.0)	13 (50.0)	5 (26.3)	
Between 1 and 3 days	2 (14.2)	6 (23.1)	5 (26.3)	
Greater than 3 days	4 (28.6)	6 (23.1)	8 (42.1)	

Numbers may not sum to total due to missing data.

Table 3: Tick bite history for people who reported itch with tick bites (N=28) and those who did not (N=31).

Characteristic	N	% Itch	Odds Ratio (CI 95%)	P Value
Tick Bites in 12 months				
0	19	31.6	1.00	
1	9	22.2	0.48 (0.08-2.95)	0.43
2	9	55.6	1.20 (0.28-5.18)	0.80
≥3	**16**	**75.0**	**6.5 (1.46-28.8)**	**0.01**
Tick Bites in 5 years				
0	9	25.0	1.00	
1-5	25	32.0	1.41(0.30-6.68)	0.66
≥ 6	**19**	**84.2**	**16.0 (2.65-96.47)**	**<0.01**

Numbers may not sum to total due to missing data.

Table 4: Skin bite reactions among people who reported itch with tick bites (N=28) and those who did not (N=31).

Characteristic	Itch with tick bite Group (no. (%))	No Itch with Tick Bite Group (no. (%))	P Value
Erythema	**26 (100.0)**	**23 (74.2)**	**<0.01**
Swelling	**22 (100.0)**	**10 (32.3)**	**<0.01**
Induration	**16 (69.6)**	**7 (22.5)**	**<0.01**
Delayed Type Hypersensitivity	**12 (63.2)**	**2 (8.3)**	**<0.01**
Pain	3 (17.6)	3 (10.0)	0.46

Numbers may not sum to total due to missing data.

Table 5: Skin bite reactions among people who reported itch with tick bites (N=28) and those who did not (N=31).

Characteristic	N	% Itch	Odds Ratio (CI 95%)	P Value
Erythema				
Yes	**49**	**46.9**	**19.17 (1.05-350.42)**	**0.05**
No	8	0.0	1.00	
Swelling				
Yes	**32**	**68.75**	**92.14 (5.08-1671.29)**	**<0.01**
No	21	0.0	1.00	
Induration				
Yes	**23**	**69.6**	**7.83 (2.30-26.65)**	**<0.01**
No	31	22.6	1.00	
Delayed Type Hypersensitivity				
Yes	**14**	**76.9**	**18.86 (3.37-105.49)**	**<0.01**
No	29	28.3	1.00	
Swelling Only				
Yes	11	63.6	3.77 (0.94-15.19)	0.06
No	41	31.7	1.00	
Induration Only				
Yes	20	0.0	0.51 (0.02-13.19)	0.69
No	32	3.1	1.00	
Past Itch				
Yes	**26**	**70.4**	**11.94 (3.25-43.89)**	**<0.01**
No	27	18.5	1.00	

Numbers may not sum to total due to missing data.

Table 6: Tick bite history for people who reported past Lyme disease (N=21) and those who did not (N=37).

Characteristic	N	% Lyme	Odds Ratio (CI 95%)	P Value
Tick Bites in 12 months				
0	16	6.0	1.00	
1	10	40.0	10.00 (0.92-108.82)	0.06
2	**11**	**45.0**	**12.50 (1.19-130.62)**	**0.04**
≥3	**13**	**53.8**	**17.50 (1.76-174.43)**	**0.01**
Tick Bites in 5 years				
0	9	0.0	1.00	
1-5	28	28.6	7.88 (0.41-151.14)	0.46
≥ 6	**16**	**75.0**	**52.78 (2.52-1104.60)**	**0.01**

Numbers may not sum to total due to missing data.

Table 7: Skin bite reactions among people who reported past Lyme disease (N=21) and those who did not (N=37).

Characteristic	N	% Lyme	Odds Ratio (CI 95%)	P Value
Erythema				
Yes	48	35.4	0.91 (0.19-4.30)	0.91
No	8	37.5	1.00	
Itch				
Yes	21	42.9	1.20 (0.37-3.87)	0.76
No	26	38.5	1.00	
Pain				
Yes	8	12.5	0.19 (0.02-1.72)	0.14
No	35	42.8	1.00	
Induration				
Yes	23	26.1	0.38 (0.12-1.21)	0.10
No	31	48.4		
Delayed Type Hypersensitivity				
Yes	12	41.7	1.30 (0.33-5.08)	0.71
No	31	35.5	1.00	
Swelling				
Yes	30	36.7	1.16 (0.34-3.96)	0.82
No	18	33.3	1.00	
Swelling Only				
Yes	9	44.4	1.47 (0.33-6.52)	0.61
No	34	35.3	1.00	

Numbers may not sum to total due to missing data.