Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original Article

Exploration of stable isotope analysis for tick host identification

Kathleen LoGiudice[a,*], Kathryn Kurchena[a,b], Katherine Christopher[a], Natasha Scott[a,c]

[a] Department of Biological Sciences, Union College, 807 Union St., Schenectady, NY 12308, USA
[b] University of Vermont, Larner College of Medicine, Burlington, VT, USA
[c] Highland Park Community School K760, Brooklyn, NY, USA

ARTICLE INFO

Keywords:
Carbon isotopes
Nitrogen isotopes
Stable isotope
Tick
Ixodes scapularis
Bloodmeal analysis

ABSTRACT

Due to the problem of tick-borne diseases, there is a need to better understand the importance of different host species in maintaining enzootic disease cycles. We explored the utility of stable isotope analysis to identify the larval hosts of questing ixodid ticks. In laboratory experiments, we used *Ixodes scapularis* and two host species that are important in the Lyme disease system in eastern North America. First, we tested how effectively a short-term dietary tracer (^{13}C in corn) was reflected in molted ticks. Second, we attempted to identify the host species (either white-footed mouse (*Peromyscus leucopus*) or eastern chipmunk (*Tamias striatus*)), based on the isotopic signature of the ticks that had fed on them.

The corn isotopic signal was easily detectable in the ticks that fed on corn-diet hosts despite the brief feeding period (96 h). However, we were not able to differentiate between flat *Ixodes scapularis* nymphs that had fed as larvae on mice vs. those fed on chipmunks. Isotopic signatures of fur from mice and chipmunks were also indistinguishable, probably due to the similar diets of these two species in the wild. We conclude that, while stable isotope analysis of ticks may not be able to distinguish between ecologically similar host species, it may be useful in sorting ticks to the level of feeding guild of the host.

1. Introduction

Tick-borne diseases are a major public health concern in many parts of the world. Despite considerable study, many aspects of basic tick ecology remain imperfectly understood, including the importance of different host species in regulating tick populations and maintaining enzootic disease cycles. A better understanding of host use may lead to ecology-based strategies to reduce human disease risk. There have been several approaches to determining host use by ixodid ticks including direct observation of infestation (Mather et al., 1989; Ostfeld et al., 1996; LoGiudice et al., 2003) and molecular methods that attempt to identify host DNA (Kent, 2009) or proteins (Laskay et al., 2013; Önder et al., 2013) in the fed or molted tick. Unfortunately each of these approaches has limitations due to bias or technical problems (Gómez-Díaz and Figuerola, 2010; Hamer et al., 2015, but see newer work such as Campana et al., 2016), exposing the need for a reliable method to identify the host that a tick fed on many months in the past.

Stable isotope analysis (SIA), a technique long used to study food webs, has recently been identified as a promising method for tick-host identification (Gómez-Díaz and Figuerola, 2010). Isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are typically used to investigate animal diets, and should allow us to distinguish carnivore from omnivore from herbivore based on the isotopic signature of tissues (Tieszen et al., 1983; Crawford et al., 2008; Martínez del Rio and Carleton, 2012). Schmidt et al. (2011) and Hamer et al. (2015) demonstrated that isotopic profiles of ticks after stadial transition reflect those of host tissues with the expected trophic enrichment in ^{15}N (McCutchan et al., 2003; Fry, 2006), suggesting that the C/N isotopic ratios of tick hosts may be distinct enough to identify the species from which a flat tick took its most recent bloodmeal. This idea assumes that 1) each host species has an isotopically unique diet in nature, and/or 2) there are mechanisms of host species' metabolism that result in differential isotopic fractionation, leading to unique isotopic ratios for different species regardless of dietary similarity. Hamer et al. (2015) analyzed the blood of wild individuals of 6 species of host and found that most of the species were isotopically distinct in both $\delta^{13}C$ and $\delta^{15}N$. They concluded that SIA could be useful for identifying the host species of flat ticks either independently or as a complement to DNA based methods. They stressed that, like molecular methods which rely on a library of markers for different wild animal species, interpretation of SIA results will depend on a thorough record of isotopic signatures from potential host species. Since these signatures can vary regionally (Cormie and Schwarcz, 1994) and with habitat type (Lavin et al., 2003), it may be necessary to create quite local libraries.

* Corresponding author.
E-mail address: logiudik@union.edu (K. LoGiudice).

http://dx.doi.org/10.1016/j.ttbdis.2017.08.010
Received 28 December 2016; Received in revised form 20 August 2017; Accepted 22 August 2017
1877-959X/ © 2017 Elsevier GmbH. All rights reserved.

In this study, we investigate the feasibility of differentiating between ticks fed on two ecologically similar species that are important hosts of blacklegged ticks (*Ixodes scapularis*). The eastern chipmunk (*Tamias striatus*) and the white-footed mouse (*Peromyscus leucopus*) are common rodents of the forests and fields of northeastern North America. Both species are opportunistically omnivorous, consuming plant based diets with seeds and grains comprising a large portion of their caloric intake and animal materials eaten irregularly according to availability (Wrazen and Svendsen, 1978; Snyder, 1982). In our laboratory experiment, animals were offered identical food choices while being parasitized by blacklegged ticks, and we explored whether there were isotopic differences in ticks that had fed on the two host species. In addition, we used a dietary tracer (^{13}C signal in corn), introduced into the diets of animals simultaneously with tick infestation, to determine if this signal would be incorporated into the ticks in the short time-frame of our experiment.

2. Materials and methods

Wild eastern chipmunks and white-footed mice were captured in Sherman traps baited with oats and sunflower seeds from 15 June 2015 through 13 July 2015 in the Albany Pine Bush Preserve (Albany County, NY, USA) and the Reist Sanctuary (Schenectady County, NY, USA), wooded areas approximately 8 km apart. Animals were sexed, weighed, and tagged, and juveniles (based on pelage coloration) and pregnant or lactating females were immediately released. All other animals were held in the laboratory for 96 h, after which they were released at the point of capture. Natural tick body burdens were very low, therefore, we infested each animal with 40–60 *I. scapularis* larvae raised from a single dog-fed female tick. Thirty chipmunks and 31 mice were held. Foods provided to animals during the captive period were stored at −80 °C, dried in a drying oven at 60 °C for 7 days and ground to a powder in preparation for stable isotope analysis. Several samples of each food were bulked and analyzed in triplicate.

During the holding period, animals were randomly assigned to be fed either a diet of apple (for hydration), sunflower seeds and walnuts *ad lib*. (standard diet), or the same diet with a restricted number of seeds and nuts and supplemented with *ad lib*. dried and fresh corn kernels (corn diet). We intended to use ticks collected within the first 24 h after capture as representative of the natural diet, however very low body burdens prevented this. Therefore, we collected fur samples from animals that produced at least 15 engorged larvae (n = 5 corn diet and n = 5 standard diet), under the assumption that the isotopic signatures in fur were representative of the natural diet, albeit one produced on a very different time scale than would be incorporated into the ticks. All animal handling and holding protocols were approved by the Institutional Animal Care and Use Committee at Union College, 807 Union St. Schenectady, NY 12308 and were in full compliance with the 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education (Sikes, 2016).

Engorged ticks were randomized by host species and diet and allowed to molt in soil cores in the field as part of a separate experiment. Molted nymphs (molting percentage = 53%) were retrieved in January 2016, 6–7 months after engorging. Upon retrieval, ticks were cleaned with DI water and frozen at minus 70 °C, dried in a drying oven for 7 days at 60 °C and prepared for stable isotope analysis; ticks were analyzed whole. The mass of a single *I. scapularis* nymph (0.07–0.1 mg) is too low to analyze with most mass spectrometers (but see Langel and Dyckmans, 2014). Therefore, they were batched in groups of 3 nymphs that had fed on the same host species on the same diet, but not the same individual (mean mass = 0.25 ± 0.02 mg SD, n = 52) or 5 nymphs fed on the same host individual (0.42 ± 0.04 mg, n = 16). This produced two separate datasets. The first dataset contained isotopic information from 52 batches of 3 ticks each in which the species and captive diet of the hosts were known. These had fed on 31 mice and 30 chipmunks. The second dataset contained 16 batches of 5 nymphs each that had fed as larvae on 7 mice and 5 chipmunks consuming either a corn or standard diet. Some animals had 2 tick samples, and in these cases we used the mean of the delta values for each animal. We obtained fur samples from 10 of these animals (5 mice and 5 chipmunks). Both datasets were analyzed with two-way ANOVA followed by Tukey's honestly significant difference tests to determine the influence of these two independent variables (host species and diet) on δ^{15}N and δ^{13}C. The datasets violated the assumptions of normality (slightly) and homoscedasticity (significantly), with significantly more variation in both δ^{15}N (F-test; $p = 0.01$) and δ^{13}C ($p = 0.0003$) in ticks that fed on corn-diet animals than on those fed on standard diet animals. This is likely due to food preference of the different species or of individual animals. Therefore, we also performed the statistical analysis on ranked data (Scheirer-Ray-Hare extension of the Kruskal Wallis test, Sokal and Rohlf, 1995), and the results were consistent with the parametric tests (See web tables and figures for non-parametric results).

Samples were analyzed at the Union College Stable Isotope Laboratory on a Thermo Delta Advantage mass spectrometer in continuous flow mode connected to a Costech Elemental Analyzer via a ConFlo IV. Reference standards (9 Sucrose [IAEA-CH-6], 13 Acetanillide, 9 ammonium sulfate [IAEA-N-2] and 10 caffeine [IAEA-600]) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. Percent C and N were calculated using an additional 3 acetanillide standards (per run) of varying mass. Corrections were done using a regression method. The combined uncertainty (analytical uncertainty and average correction factor) for δ^{13}C (VPDB) is 0.05‰ and δ^{15}N (Air) is ± 0.15‰, based on 17 Acetanillide standards over 4 analytical sessions.

3. Results and discussion

Our data confirm the results of Schmidt et al. (2011) and Hamer et al. (2015) that the isotopic signatures of molted nymphal ticks reflect the larval host diet. We took this one step further by manipulating the host diet to enrich ^{13}C and demonstrated that this enrichment is detectable up the food chain in molted ticks. In the larger dataset consisting of molted nymphs with known host species and diet, diet was a significant main effect for both δ^{13}C and δ^{15}N. The corn tracer was clearly detectable, with ticks fed on corn diet hosts significantly enriched in ^{13}C compared to ticks fed on hosts consuming a standard diet ($F(1, 48) = 104.66$, $p < 0.0001$; mean = −22.44 ± 0.11‰ SE vs. −24.22 ± 0.14‰ for corn and standard diet ticks, respectively; Fig. 1). There were no differences in δ^{13}C based on species ($p = 0.39$).

More surprisingly, δ^{15}N was also higher in animals eating corn ($F(1, 48) = 37.71$, $p < 0.0001$; Fig. 1) but there was a significant interaction between host species and diet such that ticks fed on chipmunks eating the corn diet were enriched in ^{15}N (mean = 6.71 ± 0.14‰ SE) compared to ticks fed on standard diet chipmunks and mice on either diet (4.87 ± 0.22‰, 5.57 ± 0.15‰, and 5.33 ± 0.16‰ for standard diet chipmunks, corn diet mice and standard diet mice, respectively; $F(1, 48) = 22.11$, $p < 0.0001$). This could be due to metabolic differences between the two species or to food preferences, with chipmunks eating more dried corn, which is somewhat enriched in ^{15}N over fresh corn, and mice preferring fresh corn (Table 1). Since we did not track consumption of the individual foods, we are unable to distinguish between these two possibilities.

Using the smaller dataset that paired fur samples with molted nymphs from the same individuals, ticks showed characteristic trophic enrichment in δ^{15}N over the fur from their hosts (mean enrichment 3.77 ± 0.22‰ SE, Fig. 2). This is well within the expected range from prey to consumer (2–4%; McCutchan et al., 2003; Crawford et al., 2008; Ben-David and Flaherty, 2012) and almost precisely matches the Schmidt et al. (2011) blood-tick results. Of course, fur can only be expected to give the most general indication of blood isotopic levels because of the difference in tissue turnover times (Martínez del Rio and Carlton, 2012), possible species-specific difference in blood/fur spacing

Download English Version:

https://daneshyari.com/en/article/8507392

Download Persian Version:

https://daneshyari.com/article/8507392

Daneshyari.com