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META-ANALYSIS OF CO-INFECTIONS IN TICKS

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ABSTRACT

Microbial infections typically do not occur in isolation but co-occur within diverse communities of bacteria, fungi, protozoans, and viruses. Co-infections can lead to increased disease severity, lead to selection for increased virulence, and complicate disease diagnosis and treatment. Co-infections also occur in disease vectors, and represent one source of co-infections in hosts. We examined patterns of co-infections in ticks (Order Acari), which vector diverse human and wildlife pathogens, and asked whether the frequency of microbial co-infections deviated significantly from independent associations. Most published data were from *Ixodes* species and reported infection and co-infection frequencies of *Borrelia burgdorferi* and *Anaplasma phagocytophilum*. A total of 18 datasets representing 4978 adult ticks met our criteria for inclusion in the meta-analysis. Significant deviations from independent co-infection were detected in eight of the 18 populations. Five populations exhibited a significant excess of *A. phagocytophilum*/*B. burgdorferi* co-infections, including all populations of *I. ricinus* that deviated from independence. In contrast, both populations of *I. persulcatus* and one of two populations of *I. scapularis* exhibited a significant deficit of co-infection. The single population of *I. pacificus* examined had a significant excess of co-infection. Our meta-analyses indicate that tick-borne microbes are often distributed non-randomly, but the direction of deviation was not consistent, indicating that multiple mechanisms contribute to these patterns. Unfortunately, most published studies were not designed to describe patterns of co-infection, and provided insufficient data for our meta-analysis. Future studies should more explicitly measure and report co-infections in ticks, including co-infections by endosymbionts.

Keywords: Co-infection, ticks, meta-analysis, *Ixodes*, *Borrelia burgdorferi, Anaplasma phagocytophilum*

INTRODUCTION

There is growing recognition that many microbial infections do not occur in isolation but often occur within diverse communities of bacteria, fungi, protozoans, and viruses (co-infections *aka* concurrent, dual, mixed, polymicrobial, or simultaneous infections;

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(Brogden et al., 2005)). Co-infections can lead to increased disease severity, lead to selection for increased virulence, and complicate disease diagnosis and treatment (Thomas et al., 2001; Regev-Yochay et al., 2004). With the expanding application of molecular techniques, we are continuously documenting co-infection patterns within microbial communities across many host taxa (Ley et al., 2008). For example, white-footed mice (*Peromyscus leucopus*) sampled in Minnesota and Wisconsin were highly co-infected with the tick-borne pathogens *Borrelia burgdorferi*, *Babesia microti*, and *Bartonella* spp., and no infections of *Babesia microti* alone were found (Hofmeister et al., 1998). Similarly, 26% of patients with Lyme disease (caused by *B. burgdorferi*) were co-infected with *B. microti* or *A. phagocytophilum* (Steere et al., 2003), and co-infection increases pathogenicity of Lyme disease in laboratory mice (Thomas et al., 2001). Likewise, humans from sub-Saharan Africa have been found to be co-infected by *P. malariae* and *P. falciparum* more frequently than expected by chance. Increased density of *P. falciparum* gametocytes is also positively associated with concurrent infection with *P. malariae* in subjects from multiple malaria-endemic parts of the world (Bousema et al., 2008). Understanding patterns of co-infections can contribute to our understanding of disease ecology and enhance human health.

Many of the world's most important diseases are vector-borne. Co-infections also occur in disease vectors, and represent one source of co-infections in hosts (Ginsberg 2008; Nieto and Foley, 2009). For example, *Aedes* and *Anopheles* mosquitoes can be simultaneously infected with malarial and filarial parasites (Albuquerque and Ham, 1995; Paul et al., 2002). Tsetse flies can be multiply infected with various *Trypanosoma* strains (Masiga et al., 1996; Kubi et al., 2005), and human body lice transmit a number of infections including typhus and trench fever (Goddard, 2000). Similarly, prairie dog fleas, *Oropsylla spp*., can be infected with multiple strains of *Bartonella* and *Rickettsia* (Jones et al., 2008). Fleas can also be co-infected with *Yersinia pestis*, plague, and multiple strains of *Bartonella* (Stevenson et al., 2003). *Polygenis gwyni*, a flea commonly found on rats, can also be co-infected with different strains of *Bartonella* (Abbot et al., 2007). Interactions between microbial species or strains within the vector can impact disease transmission, acquisition, and fitness of the vector, and may represent a strategy for disease control. For example, co-infection of *Anopheles* mosquitoes with *Plasmodium* strains can lead to increased virulence (Boëte and Paul, 2006). Further, a recent study proposed the introduction of a *Wolbachia* endosymbiont that shortens the lifespan of *Anopheles* mosquitoes, inhibiting complete internal development of *Plasmodium* (McMeniman et al., 2009). Thus, understanding patterns of co-infection in vector populations may help elucidate mechanisms of transmission, estimate human health risk, and potentially reveal management strategies to reduce disease in human or wildlife populations.

CO-INFECTIONS IN TICKS

Ticks (Order Acari) vector a diverse group of human and wildlife pathogens, including viral, bacterial, and protozoan disease agents (Table 1). These can coexist and interact within ticks, and and can be co-transmitted to humans and other vertebrates

Table 1
Human pathogenic viral, prokaryotic, and eukaryotic microbes identified in four widely distributed tick genera. This list is not meant to be comprehensive, but to highlight the overlap within and among tick genera. There is significant microbial diversity both within and among tick genera, which represent both hard (Ixodid) and soft (Argasid) ticks

Human pathogenic tick-borne microbes	Tick genera			
	Ixodes	*Haemaphysalis*	*Rhipicephalus*	*Ornithodoros*
Tick-borne encephalitis virus complex	x	x	x[ab]	x[ac]
Colorado tick fever virus	x	x		x
Crimean-Congo hemorrhagic fever virus	x		x	
Rickettsia	x	x	x	x[de]
Anaplasma	x	x[f]	x[f]	
Ehrlichia	x	x	x	
Borrelia	x	x[f]		x
Francisella	x	x		x[dg]
Babesia	x	x[h]	x[h]	

a. Transmission following experimental infection; b. Turell et al., 2004; c. Labuda et al., 1993; d. Unconfirmed human pathogenicity; e. Cutler et al., 2006,; f. Barandika et al., 2008; g. Noda et al., 1997; h. Garcia-Sanmartin et al., 2008. All other entries from Brown et al., 2005.

(Clay et al., 2006). For example, *Ixodes* ticks may be simultaneously infected by *Borrelia burgdorferi, Babesia microti, Anaplasma phagocytophilum, Bartonella henselae*, and other human pathogens (Goodman et al., 2005). Similarly, *Amblyomma* ticks may simultaneously harbor *Borrelia lonestari, Ehrlichia* spp., and *Rickettsia amblyommii* (Clay et al., 2008). Interest in ticks as vectors of emerging and resurgent diseases (Gubler, 1998; Gratz, 1999) has increased due to the widespread application of PCR-based techniques and the growing literature reporting co-infections in humans (Benach et al., 1985; Swanson et al., 2006). Tick-borne co-infections pose additional obstacles to clinical treatment such as increased severity and duration of illness (Krause et al., 1996; Alekseev et al., 2001; Nyarko et al., 2006), and misdiagnosis due to symptom overlap (Belongia et al., 1999).

Ticks also carry a range of vertically-transmitted (VT) endosymbionts (Niebylski et al., 1997; Noda et al., 1997; Sun et al., 2000; Grindle et al., 2003; Benoit and Yoder, 2004; Scoles, 2004; Lo et al., 2006; Morimoto et al., 2006). VT endosymbionts are often closely related to other pathogenic, horizontally-transmitted (HT) microbes. For example, avirulent VT *Coxiella, Francisella*, and *Rickettsia*-like bacteria have been identified in many tick species, and are often geographically widespread and highly prevalent within populations (Noda et al., 1997; Kurtti et al., 2002; Scoles, 2004; Goethert and Telford, 2005; Clay et al., 2008). Competition and crossover of vertebrate host immune

response may be greatest between closely related strains (Burns and Barthold, 1999; Pal et al., 2001), so that VT endosymbiont distribution could affect the prevalence of HT pathogens (Lively et al., 2005; Steiner et al., 2008). For example, infection by some Spotted Fever Group *Rickettsia* in *Dermacentor variabilis* prevents establishment and vertical transmission of related *Rickettsia* (Macaluso et al., 2002).

Vertical transmission promotes co-infection, given that newly hatched larval ticks will already be infected by one or more endosymbionts. Host feeding by larvae, nymphs, and adults can lead to the acquisition of additional microbes sequentially or simultaneously (Fig. 1). Vertebrate host community composition can also influence co-infections within ticks, through host differences in reservoir competence for tick-borne pathogens. For example, taiga ticks, *Ixodes persulcatus*, feed on over 300 species of mammals, birds, and reptiles (Filippova, 1977), and different host species may be reservoirs for

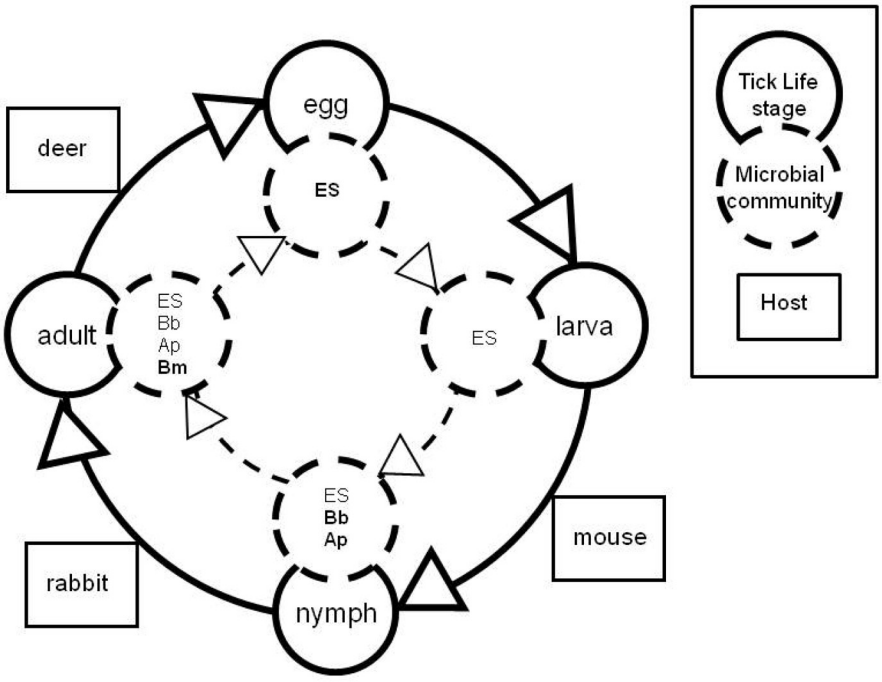

Fig. 1. Illustration of the tick life-cycle, with hypothetical tick microbial communities and hosts. After hatching from an egg, the tick (solid circle) takes a blood meal from a host (rectangle) during each life stage. Dashed circles represent the tick-borne microbial community. Newly acquired infections are bold. The endosymbiont (ES) is vertically-transmitted, while remaining microbes are acquired during host-feeding. In general, vertical transmission is rare or absent among these pathogens. (Pathogen abbreviations: Bb = *Borrelia burgdorferi*, Ap = *Anaplasma phagocytophilum*, Bm = *Babesia microti*).

different tick-borne pathogens (DeNatale et al., 2002; LoGiudice et al., 2003; Christova and Gladnishka, 2005). Nymphs, larvae, and adult ticks often feed on different host species due to differences in habitat use, microclimatic conditions, and host-seeking behavior (Randolph and Storey, 1999), leading to accumulation of more diverse microbial communities over developmental stages (Fig. 1). Moreover, ticks parasitize a small fraction of the host population, a phenomenon known as overdispersal. Tick-specific pheromones promote overdispersal by inducing ticks to feed on previously parasitized, i.e., known suitable, hosts (Norval et al., 1989). Overdispersal may promote co-infections in hosts as each additional tick bite increases the likelihood of co-infection in the host and/or pathogen transmission among co-feeding ticks (Ginsberg, 2008).

Once co-infected, interactions among these diverse microbes within their tick vector may affect transmission to humans by influencing the population density of pathogens, and the fitness of ticks. Facilitative microbial interactions could raise human health risk if the acquisition, establishment, or maintenance of a secondary infection is promoted in the tick by a previously acquired pathogen, increasing the chances of co-transmission. Conversely, competitive interactions might prevent the establishment or maintenance of multiple pathogens, decreasing the likelihood of co-infections arising from single tick bites. Microbial interactions within ticks may be a more important determinant of co-infection risk than with other blood-feeding vectors. Ticks are relatively long-lived, with longer periods of time between blood meals compared to mosquitoes, lice, mites, fleas, and sand flies (Balashov, 1984; Randolph, 2004). Therefore, microbial co-infections and tick survival must be sustained over a much longer temporal scale.

OBJECTIVES

The goal of this paper is to review the published literature for reports of co-infections in ticks. When the available data are sufficient, we ask whether there is evidence of non-random patterns of co-infection (e.g., facilitation or inhibition) of microbes infecting ticks (see also Ginsberg, 2008; Nieto and Foley, 2009). Our results will serve as a catalyst for further investigation of the dynamics of co-infections of tick-borne pathogens. Our results also point to improved methods for sampling, microbial diagnostics, reporting and statistical analysis. In addition to raising awareness and reducing human disease, increased understanding of microbial communities within ticks may lead to novel controls of tick-borne and other arthropod-vectored diseases. This will be especially important if the spatial distributions of ticks and tick-borne pathogens continue to shift (Fraenkel et al., 2002; Danielova et al., 2006; Stunzner et al., 2006), potentially producing novel microbial communities, microbe-tick (Demma et al., 2005), or host-microbe-tick combinations.

METHODS

We surveyed the literature for reports of infection and co-infection in ticks using ISI's Web of Knowledge. We searched combinations of the terms *tick*, *co-infection*, *parasite*, *pathogen*, and *symbiont*. We incorporated wildcard characters for the terms and also

manually reviewed the titles of the cited literature of most, if not all, relevant papers. Co-infections among viral, bacterial, and eukaryotic human pathogens have been reported in several genera of ticks, including hard (Ixodidae) and soft (Argasidae) ticks.

We included only PCR-based studies of at least 100 adult ticks from a single sampling location no larger than single counties or equivalent areas. Studies identifying distribution and prevalence of pathogens among tick populations typically sample between 50–500 ticks per site or study. With sample size $n \geq 100$, there is good statistical power for the evaluation of interactions between moderately prevalent (10–70%) pathogens such as *A. phagocytophilum* and *B. burgdorferi*. Clay et al. (2006) found that sample sizes of 1000 or more may be required to detect interactions when both microbes are less common (≤5%).

Little, if any, co-infection data has been published for the vast majority of tick-associated microbial communities. Reports of *Ixodes* tick infection by *Anaplasma phagocytophilum* and *Borrelia burgdorferi sensu lato* represented the overwhelming majority of these studies (see also Nieto and Foley, 2009). Therefore, we restricted our meta-analysis to these studies.

We applied a G-test for independence ($\alpha = 0.05$) with William's correction (Sokal and Rohlf, 1981) to these datasets to test the null hypothesis that the likelihood of tick infection by *A. phagocytophilum* or *B. burgdorferi* was independent of infection by the other microbe. Expected co-infection frequencies are obtained by obtaining the product of the individual infection prevalences. For example, in a tick population with 50% prevalence of both *A. phagocytophilum* and *B. burgdorferi,* 25% of ticks are expected to be co-infected. Further, to better compare our results with Nieto and Foley (2009), we also calculated odds ratios as described in their paper. Odds ratios greater than one indicate an excess of co-infections, while those less than one indicate a deficit of co-infections, relative to the expected frequency.

Distinct datasets from individual publications were analyzed separately. Datasets from different sampling areas were analyzed separately, as were datasets collected from individual sites in different years. Studies that ambiguously defined any of the infection classes (uninfected, *A. phagocytophilum* only, *B. burgdorferi* only, or co-infected) were excluded in order to prevent the misinterpretation of infection frequencies.

RESULTS

A total of 18 datasets from 15 publications met our criteria for inclusion in our analyses (Table 2). Four species of the *Ixodes ricinus* species complex were represented: *I. pacificus, I. persulcatus, I. ricinus*, and *I. scapularis*. The 18 datasets examined represented a total of 4978 adult ticks. Overall, 31.6% (1573 ticks) were infected with *B. burgdorferi* and 14.4% (716) were infected with *A. phagocytophilum*. Prevalence of *B. burgdorferi* among *Ixodes* populations ranged from 11.6–67.3% while *A. phagocytophilum* prevalence ranged from 6.2–53.0%. A total of 5.4% (268) of all ticks were co-infected.

Significant deviations from random likelihood of co-infection were detected in eight of the 18 (44.4%) tick populations (Table 2). The single *I. pacificus* population studied

Table 2

Studies reporting infection status for adult ticks of the *Ixodes ricinus* species complex with *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum*. Five tick populations exhibited a significant excess of co-infections under the null hypothesis of independent infection of the two pathogens. Three populations exhibited a significant deficit of co-infections. Individual pathogen counts exclude dually infected ticks. Arrows indicate the direction of significant deviations from independent infection. Odds ratios were calculated as in Nieto and Foley (2009). P-values are derived from G-tests for independence (Sokal and Rohlf, 1981)

Tick species	Not infected	*B.b*+ only	*A.p*+ only	Co-infected	Odds ratio	P-value	Author
I. scapularis	37	71	27	53	1.02	0.94	Schauber et al., 1998
I. scapularis	93	114	13	9	0.56	0.21	Chang et al., 1998
I. scapularis	102	13	64	12	1.47	0.38	Courtney et al., 2003
I. scapularis	21	26	27	26	0.78	0.53	Schwartz et al., 1997
I. scapularis	42	26	13	19↑	2.36	<0.05	Schwartz et al., 1997
I. scapularis	31	53	11	5↓	0.266	<0.05	Steiner et al., 2008
I. scapularis	59	27	6	8	2.91	0.07	Steiner et al., 2008
I. scapularis	46	37	11	6	0.68	0.48	Varde et al., 1998
I. pacificus	692	36	40	8↑	3.84	<0.01	Holden et al., 2003
I. ricinus	53	21	23	15	1.65	0.24	Christova et al., 2001
I. ricinus	312	28	63	21↑	3.71	<0.001	Stanczak et al., 2002
I. ricinus	186	33	59	25↑	2.39	<0.01	Stanczak et al., 2004
I. ricinus	200	56	16	8	1.79	0.23	Skotarczak et al., 2003
I. ricinus	174	96	7	18↑	4.66	<0.001	Alekseev et al., 2001
I. ricinus	135	45	13	5	1.15	0.80	Koci et al., 2007
I. persulcatus	105	202	5	24	2.50	0.053	Alekseev et al., 2001
I. persulcatus	322	166	31	3↓	0.19	<0.001	Cao et al., 2003
I. persulcatus	347	255	19	3↓	0.21	<0.01	Cao et al., 2003

showed an excess of co-infections. Two of the three *I. persulcatus* populations exhibited a significant deficit of co-infections, while three of six *I. ricinus* populations showed a significant excess of co-infections. One of seven *I. scapularis* populations showed a significant excess of co-infections while one showed a significant deficit. The remaining ten datasets showed no significant deviation from random co-infection prevalence.

Thus, nearly half of the studies meeting our criterion for inclusion exhibited a statistically significant deviation from independence. However, the direction of the deviations was not consistent, as odds ratios ranged from 0.19–4.66.

DISCUSSION

Investigation of tick-borne pathogens has generally focused on prevalence of specific horizontally-transmitted (HT) pathogens among, within, and across populations of single tick species (reviewed in Swanson et al., 2006), mammal reservoir species (Chris-

tova and Gladnishka, 2005), and humans (Mitchell et al., 1996), based on serological or PCR detection. A majority of published reports focus on the genus *Ixodes*, reflecting its importance as the principle vector of the disease agents of Lyme borreliosis, anaplasmosis, and tick-borne encephalitis (Barbour and Fish, 1993). Studies on the distribution of these disease agents, as well as *Babesia, Bartonella,* and *Rickettsia* species, have estimated both prevalence and disease risk across the global range of *Ixodes* ticks. Site-specific data are essential for determining relevant disease agents, identifying reservoir species, and assessing human health risk. However, few studies have been designed to describe co-infection patterns within ticks, despite the frequent co-occurrence of multiple pathogens and endosymbionts within individual ticks.

In the meta-analysis presented here, 44% of the *Ixodes* tick populations (8 of 18) meeting our criteria for inclusion for analysis significantly deviated from expected co-infection frequencies under the assumption of independent infection of *A. phagocytophilum* and *B. burgdorferi*. It will be informative to obtain data from a wider range of tick and microbial species.

I. ricinus populations only exhibited significant excesses of co-infections, as did the single *I. pacificus* population analyzed, a pattern consistent with microbial facilitation. In contrast, *I. persulcatus* populations only exhibited significant deficits of co-infections, a pattern consistent with interspecific competition or inhibition between pathogens (Gotelli, 2000). However, *I. scapularis* populations exhibited both significant excess and significant deficit of co-infections, indicating that there are multiple mechanisms behind these patterns. Our results therefore suggest that a disproportionate likelihood of co-infection by *A. phagocytophilum* and *B. burgdorferi* commonly occurs in natural tick populations.

Since the submission of this paper, results of another meta-analysis of co-infection of *A. phagocytophilum* and *B. burgdorferi* in *Ixodes* were published by Nieto and Foley (2009). While the overall goals were similar, there were substantial differences in methodology, inclusion criteria, and statistical analysis that complicate direct comparison. Our inclusion criteria were more stringent, based on a priori power analysis (Clay et al., 2006). For example, we included only studies that had a minimum of 100 ticks sampled. Further, we analyzed only unengorged adults, ensuring that all individuals obtained two blood meals and had similar chances for co-infection. We also did not combine data sets across sites, years, or life-stages. Pooling infection data from temporally or spatially distinct tick populations can produce spurious and misleading results, especially when individual pathogen prevalence is heterogeneous through space or time (e.g., analysis of Courtney et al., 2003). However, Nieto and Foley (2009), included a large amount of data on co-exposure of many vertebrate host species and genera, including humans, as well as data on larval and nymphal ticks. For the 13 datasets analyzed here which were also analyzed by Nieto and Foley (2009), we found general qualitative agreement of odds ratios (except for Courtney et al., 2003) even though the exact data included were different. Taken together, these results provide robust evidence for the occurrence and non-independence of co-infection in *Ixodes* ticks (Ginsberg, 2008; Nieto and Foley, 2009).

Our data demonstrate that ticks harbor multiple pathogens, and that these microbes are often distributed non-randomly. However, multiple mechanisms can cause these patterns. Both facilitative and competitive microbial interactions have been observed in vitro, in ticks, and in vertebrate hosts. For example, Levin and Fish (2001), found mutual interference of transmission efficiency of *A. phagocytophilum* or *B. burgdorferi* when uninfected *I. scapularis* fed on co-infected *Peromyscus leucopus*. However, they also found that previous infection with either pathogen did not affect the efficiency with which *I. scapularis* nymphs acquired the other pathogen (Levin and Fish, 2000). Transmission interference in both ticks and reservoir hosts has been observed for other tick-borne microbe pairs. For example, de la Fuente et al. (2002) identified two pathogenic strains of *Anaplasma marginale* (a rickettsial pathogen infecting cattle) that exhibit complete mutual exclusion in tick cell lines. Moreover, cattle inoculated with identical doses of both pathogen strains invariably became infected with only a single strain. Similarly, prior infection with *Rickettsia montana* (now *R. montanensis*) prevented vertical transmission of nonpathogenic *R. rhipicephali* in a challenge experiment in *Dermacentor variabilis* ticks. Only *R. montana* was found infecting larval progeny (Macaluso et al., 2002). However, the pattern of inhibition found in these studies is in contrast to the results of our meta-analysis. We found that the majority of significant deviations were an excess of co-infections, suggesting a predominance of facilitation rather than inhibition. The variation between the results of laboratory studies and observed field patterns suggests that factors such as tick host-seeking behavior, host immunity, and host community composition may also influence co-infection status.

The goal of most studies reviewed here was not to quantify patterns of co-infection. In fact, most studies provided insufficient data for our meta-analysis. But in light of the generality and health consequences of co-infection, greater attention should be given to accurate measurement of co-infection in ticks. We have identified several factors limiting rigorous analysis of deviations from random patterns of co-infection: small sample sizes, lack of quantification of co-infection classes, pooling ticks from multiple sites or years, and failure to determine or report endosymbiont infections. Co-infection with non-pathogenic microbes may form the largest class of co-infected ticks (see Clay et al., 2008), but they are generally not evaluated. These factors limit our and other researchers' abilities to rigorously test for deviation from independent co-infection rates. Evaluation of co-infection status requires use of multiple specific probes rather than single probes that identify specific microbes but do not reveal the presence of other microbes. Identification of novel microbes may require other approaches, such as microscopy and 16s sequencing (Beninati et al., 2004; Klyachko et al., 2007; Clay et al., 2008).

Two important features of co-infection, in addition to presence/absence data, are microbial densities and localization within the tick. PCR-based studies have determined co-infection prevalence in whole ticks without considering relative microbial densities and potential competitive exclusion from specific tissues or organs that increase likelihood of transmission (e.g., ovaries, salivary glands). For example, *Trypanosoma cruzi*, the agent of Chagas disease, is carried by the kissing bug, *Triatoma brasiliensis*. Two strains of *T. cruzi* have been shown to interfere with each other's growth in vitro and to

segregate within different vector tissues (Araujo et al., 2007). Quantitative PCR (QPCR) techniques allow for the highly sensitive quantification of microbes and can advance understanding of transmission success, microbial interactions, and effect on vector fitness (Soares et al., 2006; Zhong et al., 2007). Fluorescent in-situ hybridization (FISH) represents another technique for investigating co-infection. For example, FISH analysis of the vertically-transmitted *Coxiella* endosymbiont of *Amblyomma americanum* ticks demonstrates that *Coxiella* is localized not only in the ovaries, as expected, but also in salivary glands, suggesting the possibility of horizontal transmission (Klyachko et al., 2007). We can apply these tools to questions not only about microbial density and localization in co-infected ticks, but also about differences among sexes, developmental stages, populations, and species of ticks, providing further insights into disease dynamics and human health risk.

Increasing attention is being paid to understanding how infection with multiple pathogens affects vector-borne disease in human and wildlife populations and communities. Progress will depend not only on knowledge of the ecology and life-history of the host and vector, but also of the full spectrum and outcome of interactions between microorganisms. Microbes can interact positively (facilitation) or negatively (competition), leading to changes in infection prevalence in host or vector populations. Because previous studies and our meta-analysis demonstrate inconsistent patterns of co-infection in ticks, including both facilitation and inhibition between tick-borne microbes, additional field and laboratory research is necessary to understand the mechanisms behind the observed patterns. Co-infections in humans, domestic animals, and wildlife raises additional complications in diagnosis and treatment, including symptom overlap, altered pathology, and non-additive microbial interactions. Co-infections in humans in particular likely reflect bites from co-infected ticks rather than multiple sequential bites from singly-infected ticks (Ginsberg, 2008).

Insights from our meta-analysis of microbial co-infections in ticks may be relevant to other human disease vectors, such as mosquitoes, lice, and fleas, which also host diverse microbial communities. Understanding how host diversity, vector ecology, and microbial interactions determine co-infection rates will enhance disease ecology and human health. However, before detailed studies of mechanisms, the first step is to more rigorously test for and quantify patterns of co-infection in ticks and other vectors.

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