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MAJOR ARTICLE

Beyond Human Babesiosis: Prevalence and Association of *Babesia* Coinfection with Mortality in the United States, 2015–2022: A Retrospective Cohort Study

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Background. The prevalence of *Babesia* coinfecting tick-borne zoonoses and mortality outcomes are not fully elucidated. The objective of the present study was to determine babesiosis coinfection prevalence rates and estimate the association with severe disease and mortality.

Methods. We queried the TriNetX database between 2015 and 2022 for patients with babesiosis. The prevalence of *Babesia* coinfecting tick-borne zoonoses was estimated. The analysis focused on babesiosis coinfection with *Borrelia burgdorferi*, ehrlichiosis, and anaplasmosis. The exposure was coinfection, and the control group was the *Babesia*-only group. The primary outcome was 90-day mortality from the diagnosis of *Babesia*. Secondary outcomes were prevalence of coinfection, association of coinfection with acute respiratory distress syndrome, multiorgan failure, and disseminated intravascular coagulation. A multivariable logistic regression model was employed to estimate the disease severity and mortality risk associated with coinfections.

Results. Of the 3521 patients infected with *Babesia*, the mean age (SD) was 56 (18) years, 51% were male, and 78% were White. The frequency of overall malignancies, lymphomas, and asplenia was 19%, 2%, and 2%, respectively. Temporal distribution of coinfections followed the overall babesiosis pattern, peaking in the summer months. The prevalence of 1 or more coinfections was 42% (95% CI, 40%–43%). The rate of coinfection with *Borrelia burgdorferi* was the highest at 41% (95% CI, 39%–42%), followed by ehrlichiosis at 3.7% (95% CI, 3.1%–4.4%) and anaplasmosis at only 0.3% (95% CI, 0.2%–0.6%). Doxycycline was more likely to be prescribed in the coinfection group than the *Babesia*-only group (25% vs 18%; $P < .0001$). Overall, 90-day mortality was 1.4% (95% CI, 1.0%–1.8%). After adjusting for potential confounding factors, compared with the babesiosis-only group, the likelihood of 90-day mortality was lower in the coinfection group (adjusted odds ratio, 0.43; 95% CI, 0.20–0.91). Severe disease did not differ significantly between the 2 groups.

Conclusions. In this extensive study of >3000 patients with babesiosis in the United States, 4 in 10 patients had coinfecting tick-borne zoonoses. The prevalence rates of coinfection were highest with *Borrelia burgdorferi,* followed by ehrlichiosis, and lowest with anaplasmosis. Coinfection with other tick-borne infections was not associated with severe disease. It is plausible that this finding is due to the likelihood of treatment of coinfections with doxycycline. Future studies are needed to investigate the possible therapeutic benefits of doxycycline in babesiosis patients as, to date, no trials with doxycycline have been conducted in human patients with *Babesia* infections.

Keywords. anaplasmosis; babesiosis; *Borrelia burgdorferi*; ehrlichiosis; tick-borne diseases.

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Human babesiosis is a tick-borne illness caused by the Apicomplexan intraerythrocytic parasites known as *Babesia* spp. [1]. Six different *Babesia* species, 3 in the United States alone, have been confirmed as human pathogens. These include *Babesia crassa*–like agent, *Babesia divergens, Babesia duncani, Babesia microti, Babesia motasi,* and *Babesia venatorum* [1]. Human babesiosis prevalence in the United States is on the rise, partly due to climate change influencing the distribution and population of vectors, and the predominant species is *Babesia microti,* which is endemic in the northeastern and northern Midwestern region [1–3]. *Babesia microti* is transmitted by the blacklegged tick vector *Ixodes scapularis,* although other tick species are vectors for other *Babesia* spp. [4, 5].

Individuals with cellular immunodeficiency such as functional or anatomic asplenia and the elderly tend to have more severe disease and mortality, and among survivors, babesiosis complications are associated with a higher health burden including chronic fatigue, renal failure, and congestive heart disease, among others [3, 6, 7]. Clinical presentation can vary significantly, ranging from asymptomatic, mild disease to death via multiorgan dysfunction and depending on the degree of immunocompromise in the affected individual [4].

In the case of confirmed diagnosis of babesiosis, testing for other tick-borne illnesses such as *Borrelia burgdorferi* (the bacterium that causes Lyme disease), anaplasmosis, ehrlichiosis, hard-tick relapsing fever (caused by *Borrelia miyamotoi*), and sometimes Powassan virus disease is often a common practice as the *Ixodes scapularis* tick vector can carry and transmit multiple organisms [5, 8]. In >16 000 ticks collected from the entire United States that underwent molecular testing for pathogens, *Borrelia burgdorferi* was detected in 20% of *Ixodes scapularis* adult ticks, 11% of nymphs, and 5.1% of larvae [9]. The presence of *Anaplasma phagocytophilum* and *Babesia microti* was detected in 4% and 2% of *Ixodes scapularis* ticks, respectively. Nearly 1% of tested ticks were coinfected with *Anaplasma phagocytophilum* and *Borrelia burgdorferi*; these accounted for the most coinfection. The prevalence of triple infections of *Borrelia burgdorferi, Anaplasma phagocytophilum,* and *Babesia microti* was only 0.1%. However, in the northeastern United States, the coinfection rate in tick vectors reached 28% of ticks tested [10], with a median range of 2%–16% and 0%–19% for adult and nymphal *Ixodes* ticks, respectively [11–13]. The most commonly reported coinfection was *Borrelia burgdorferi* with either *Anaplasma phagocytophilum* or *Babesia microti*.

Globally, studies have reported varying rates of tick-borne disease co-exposure in the human population [14]. In the United States, serological evidence has shown that 54% of patients with babesiosis test positive for immunoglobulin (Ig) G and IgM antibodies to spirochetes causing Lyme disease [15]. Furthermore, 24% of babesiosis-associated hospitalizations list Lyme disease as a codiagnosis [16]. Despite the reported high prevalence of coinfecting tick-borne zoonoses, disease severity and the mortality risk of babesiosis coinfection need further characterization [11]. Various studies have explored the prevalence and impact of babesiosis-associated coinfection [17–20]. Previous reports of concurrent human Lyme disease and babesiosis suggest that coinfection may exacerbate illness [20–22]. For example, 50% of patients with concurrent Lyme disease and babesiosis were symptomatic for 3 months or longer compared with 4% of patients with Lyme disease alone [20]. These patients experienced more symptoms and a more persistent episode of illness than did those experiencing *Babesia* infection alone. In contrast, there is no evidence that *Babesia* infection or anaplasmosis enhances the dissemination of *B. burgdorferi* into the joint, nerve, or heart tissue [17]. Likewise, animal studies have provided mixed findings with respect to the association of coinfection with disease dissemination.

Some of the coinfection studies have been limited by small sample sizes. The hypothesis of the present study is that individuals with *Babesia* who are coinfected with other tick-borne infections have severe disease and higher mortality risk. The objective of this study was to characterize babesiosis coinfection prevalence rates and estimate severe disease and mortality outcomes using a large diverse representative sample size of the US population.

METHODS

Data Source

We obtained all cases of babesiosis using the International Classification of Diseases, 10th Revision (ICD-10), code B60.0 from the TriNetX database between 1980 and 2023. The data used in this study were collected on August 25, 2023, from the TriNetX Research Network. TriNetX operates as a federated, multi-institutional health research network, aggregating de-identified data from Electronic Health Records across a diverse range of health care organizations [23]. This network includes academic medical centers, specialized physician practices, and community hospitals, representing >250 million patients from >120 health care organizations [23]. As a federated network, TriNetX received a waiver from the Western Institutional Review Board (IRB) as only aggregated counts and statistical summaries of de-identified information were used; no protected health information was received, and no study-specific activities were performed in this retrospective analysis. This report follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines for reporting observational studies in epidemiology [24].

To reduce the risk of misclassification due to the differences between ICD-9 and ICD-10 codes in identifying *Babesia* cases, we excluded all ICD-9 cases, which is equivalent to data before October 1, 2015, as the ICD-10 came into effect in October of 2015 [25]. The remaining sample size consisted of 3521 individuals (Figure 1). We extracted demographics directly from the database including age in years, sex, race/ethnicity, and obesity (body mass index in kg/m^2 of 30 and above). Next, we extracted antimicrobial treatment types including azithromycin and atovaquone, clindamycin, quinine, and doxycycline using RxNorm codes. As presented in Supplementary Table 1, we extracted potential confounding comorbidities (congestive heart failure, chronic obstructive pulmonary disease, diabetes, hypertension, chronic kidney disease, all malignancies, lymphoma, rheumatoid arthritis, obesity, HIV, depression) and surrogate markers of babesiosis severity (anemia and blood transfusion), as well as additional factors known to influence severe babesiosis (asplenia). Of note, we also extracted parasitemia density, which we could not use for analysis as few records were available.

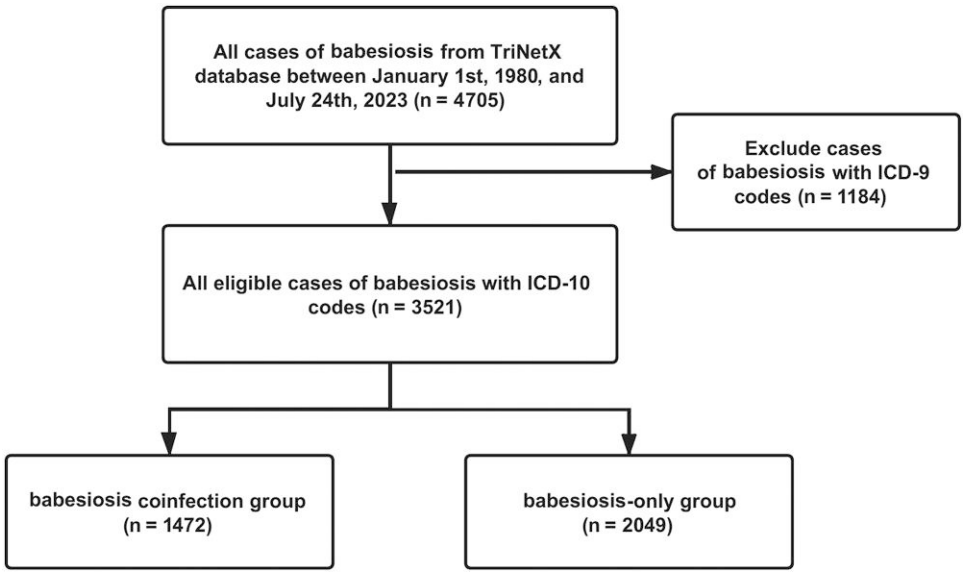

Figure 1. Study flowchart. Abbreviation: ICD-9/10, International Classification of Diseases, 9th/10th Edition.

Coinfections were defined as babesiosis infection (ICD-10: B60.0) with 1 or more additional tick-borne infections: *Borrelia burgdorferi*, ehrlichiosis, and anaplasmosis [26]. The coinfection group was created by the authors using the ICD-10 codes for Lyme disease (A69.20), ehrlichiosis (A77.40), and anaplasmosis (A79.82). A complete list of ICD-10 codes including potential confounding factors and other secondary outcomes can be found in Supplementary Table 1.

Statistical Analysis

On the basis of previously published mortality data among babesiosis patients [27] and with a sample size of 3521 patients, we consistently had sufficient power (>0.90) to detect the effect size (odds ratio) for mortality, ranging from 0.30 to 0.60. A power analysis was conducted using PASS, version 12 (NCSS, Kaysville, UT, USA) [28, 29]. Details of the power analysis are provided in Supplementary Text 1. Data were summarized using means and SDs for continuous variables. Categorical variables were summarized using frequency distributions, reporting numbers and percentages for each variable.

The primary outcome was a 90-day mortality rate comparison between coinfecting tick-borne zoonoses and the *Babesia*-only group. The rationale of 90-day mortality stems from a babesiosis and Lyme disease study that demonstrated that symptoms in coinfected patients lasted >3 months; spirochete-specific DNA was detected at a median of 91 days in coinfected patients [20]. However, as bloodstream infection–attributable death rates decay significantly over the first 2 weeks following infection, 30- rather than 90-day composite end points have been proposed [30]. Therefore, 30-day mortality was also estimated in a post hoc analysis.

Secondary outcomes were mortality risk ratio of the coinfected group vs the *Babesia*-only group in regard to acute respiratory distress syndrome (ARDS), multiorgan failure (MOF), and disseminated intravascular coagulation (DIC). Multivariable logistic regression models were conducted while adjusting for age, sex, asplenia, congestive heart failure, chronic obstructive pulmonary disease, diabetes, hypertension, chronic kidney disease, malignancy, lymphoma, rheumatoid arthritis, obesity, depression, blood loss anemia, and blood transfusion.

Because the association of babesiosis with severe disease has been shown to be modified by asplenia and anemia severity [3], we tested for potential interactions of babesiosis coinfection with asplenia and anemia severity in the regression analysis. Prevalence and associated 95% CIs were estimated using an exact binomial test.

To determine the temporal association between frequencies of babesiosis cases, we fitted generalized linear mixed-effects models assuming a Poisson distribution with log link function. We fitted time (from 2015 through 2022). A log-linked linear fit with time was estimated as $\log(\mu) = \beta_0 + \beta\,(T)$, where μ was the expected number of babesiosis cases, T was time, and β_0 and β were model parameters. All statistical analysis and figures were created using R statistical software (R Team, Vienna, Austria). Statistical significance was set at <.05.

RESULTS

A total of 3521 patients were analyzed. Table 1 shows a demographic summary of the study cohort. The mean age of the study participants (SD) was 56 (18) years, 51% were male, and the majority of the patients were White (78%), followed by

Blacks and Asians (2% each). Regarding the frequency of coinfection, 41% were coinfected with *Borrelia burgdorferi,* 4% with ehrlichiosis, and 0.3% with anaplasmosis (Figure 2). In terms of comorbidities, 16% of patients were obese, 2% had asplenia, 11% had rheumatoid arthritis, 18% had chronic obstructive pulmonary disease, 42% had hypertension, 14% had diabetes, and 0.3% had HIV. The overall malignancy rate was 19%, and 2% had lymphoma (0.34% Hodgkin's and 1.6% non-Hodgkin's). Over three-quarters of *Babesia* patients resided in the Northeastern United States and 9% in the Midwestern region, 8% in the Southern region, and 3% in the Western region. There was a statistically significant upward slope of the generalized linear model with dependency on time of the temporally averaged babesiosis cases over the 8-year interval in the United States (slope of 0.082, corresponding to an exp [0.082 = 9% increase in babesiosis per year between 2015 through 2022]; $P < .0001$; slope standard error = 0.009) (Figure 3). Seasonality of cases was observed, with higher rates of cases observed between June and September (Supplementary Figure 1).

Next, we compared the above sociodemographic and comorbidity distribution between the coinfection and *Babesia*-only groups. The *Babesia*-only patients were older (58 years vs 54 years), more likely to be male than female (55% vs 46%), more likely to have anatomical asplenia (2.5% vs 1.4%), chronic kidney disease (10% vs 8%), and congestive heart disease (11% vs 8%), and more likely to be treated with atovaquone (44% vs 39%) and azithromycin (52% vs 47%). Conversely, the babesiosis-only group was less likely to be treated with doxycycline (18% vs 25%) and less likely to be diagnosed with rheumatoid arthritis than the coinfection group.

Next, the multivariable logistic regression model was fitted to estimate the risk of mortality between those with coinfection and those without coinfection. In the full adjusted model, the likelihood of mortality was lower in the group of patients with coinfections (adjusted odds ratio [aOR], 0.43; 95% CI, 0.20–0.92) (Table 2, Figure 4A). When we limited coinfection to only *Borrelia burgdorferi*, the association was similar to the primary analysis of any coinfection (Figure 4B). However, due to the small sample size, no association was observed when an analysis was conducted between coinfection with ehrlichiosis (n = 131) and anaplasmosis (n = 11) (Figure 4C and D). In sensitivity analysis of 30-day mortality, although in a univariate logistic regression model coinfection was associated with lower mortality (OR, 0.40; 95% CI, 0.17–0.94) (Supplementary Figure 2), in the fully adjusted multivariable logistic model the association did not reach statistical significance (aOR, 0.62; 95% CI, 0.26–1.50).

Next, we estimated the association between coinfection status and secondary outcomes: acute respiratory distress syndrome, multiorgan failure, and disseminated intravascular coagulopathy. These results are summarized in Figure 5A–C. There was no association between coinfection status and acute respiratory distress syndrome (aOR, 1.56; 95% CI, 0.68–3.56), multiorgan failure (aOR, 0.82; 95% CI, 0.65–1.05), or disseminated intravascular coagulopathy (aOR, 0.99; 95% CI, 0.35–2.70).

DISCUSSION

In the present study of >3000 babesiosis patients, nearly 4 in 10 patients with *Babesia* had coinfecting tick-borne zoonoses, including *Borrelia burgdorferi*, ehrlichiosis, and anaplasmosis. This study does not support our hypothesis that *Babesia* patients coinfected with other tick-borne pathogens have a higher mortality risk. Also, this study does not specifically support that coinfected patients have a higher severity of disease. The observed association was not cofounded by major chronic comorbidities.

Studies investigating the effect of babesiosis coinfections have reported conflicting findings [18–20]. Mareedu and colleagues characterized risk factors for severe infection and hospitalization among babesiosis patients in northern Wisconsin [18]. They found an overall coinfection rate of 37%, with *Borrelia burgdorferi* documented as the highest rate of coinfection at 30%, followed by anaplasmosis at 4.5%, and both *Borrelia burgdorferi* and anaplasmosis at 2.3%. Our findings are in agreement with those of Mareedu et al., showing similar coinfection prevalence and that coinfection did not lead to higher severity of disease. In their study, coinfection with *Borrelia burgdorferi* or anaplasmosis was associated with a 27% lower risk of hospitalization (risk ratio, 0.73; 95% CI, 0.53–0.99; $P = .03$) [18]. The frequency of disease severity and duration of antibiotic treatment were similar between the babesiosis-only and coinfection groups. It was postulated that concurrent use of doxycycline (and other Lyme disease treatment) could have therapeutic benefit in *Babesia* infection, although such a therapeutic effect has not been elucidated in clinical trials. Additionally, another study found no association between co-exposure to *B. burgdorferi* and *B. microti* and increased Lyme disease severity [17]. Conversely, a study based in Rhode Island and Connecticut found that symptom quantity and duration were increased in patients with coinfection with babesiosis/Lyme disease compared with patients with either babesiosis or *Borrelia burgdorferi* alone [20].

The pathophysiological mechanisms for the lack of severe disease in patients with *Babesia* coinfection are not fully elucidated. Murine models of concurrent *Borrelia burgdorferi* and *Babesia microti* have been inconclusive. In a murine model study by Moro et al., the severity of disease from coinfection was strain dependent; no differences in severity of symptoms were found in coinfected C3H/HeJ mouse cohorts, but coinfected BALB/c mice had a significant increase in arthritis severity at day 30 [31]. In the murine model strain that demonstrated increased disease severity in the coinfected

Table 1. Baseline Characteristics of Babesiosis Patients, Overall and According to Coinfection Status

Characteristic	Overall (n = 3521)	Coinfection Group (n = 1472)	Babesiosis-Only Group (n = 2049)	*P* Value
Age, mean (SD), y	56 (18)	54 (19)	58 (18)	<.0001
Parasitemia, mean (SD)[a]	2.5 (3.6)	2.5 (4.0)	2.5 (3.3)	.94
Male sex, No. (%)	1793 (51)	672 (45.7)	1121 (54.7)	<.0001
Race, No. (%)				.13
White	2753 (78)	1150 (78.2)	1603 (78.1)	
Asian	87 (2.0)	37 (2.5)	50 (2.4)	
Black	78 (2.0)	24 (1.6)	54 (2.6)	
Native American	3 (0.1)	0 (0.0)	3 (0.2)	
Unknown	591 (17)	259 (17.6)	332 (16.2)	
Region, No. (%)	…	…	…	.001
Northeast	2733 (78)	1153 (78.3)	1580 (77.1)	
Midwest	333 (9.0)	122 (8.3)	211 (10.3)	
South	294 (8.0)	113 (7.7)	181 (8.8)	
West	118 (3.0)	68 (4.6)	50 (2.4)	
Unknown	43 (1.0)	16 (1.09)	27 (1.32)	
Comorbidities, No. (%)	…	…	…	
Obesity	567 (16)	230 (15.6)	337 (16.4)	.54
Asplenia	71 (2.0)	20 (1.36)	51 (2.5)	.03
Rheumatoid arthritis	391 (11)	197 (13.4)	194 (9.47)	.0003
Any cancer	650 (18.5)	260 (17.7)	390 (19.0)	.32
Lymphoma	83 (2)	27 (1.8)	56 (2.73)	.10
Hodgkin's lymphoma	12 (0.34)	6 (0.41)	6 (0.29)	.78
Non-Hodgkin's lymphoma	58 (1.6)	18 (1.2)	40 (2.0)	.12
HIV	10 (0.3)	6 (0.41)	4 (0.20)	.40
Chronic liver disease	427 (12)	182 (12.4)	245 (12.0)	.75
Chronic kidney disease	331 (9.0)	119 (8.1)	212 (10.3)	.03
Diabetes	492 (14)	202 (13.7)	290 (14.2)	.75
Chronic obstructive pulmonary disease	649 (18)	269 (18.3)	380 (18.5)	.87
Hypertension	1464 (42)	555 (37.7)	909 (44.4)	<.0001
Congestive heart failure	345 (10)	123 (8.4)	222 (10.8)	.02
Antimicrobials, No. (%)	…	…	…	
Atovaquone	1479 (42)	570 (38.7)	909 (44.4)	.001
Azithromycin	1752 (50)	693 (47.1)	1059 (51.7)	.01
Clindamycin	487 (14)	219 (14.9)	268 (13.1)	.14
Quinine	108 (3.0)	35 (2.38)	73 (3.56)	.56
Doxycycline	723 (21)	361 (24.5)	362 (17.7)	<.0001

Obesity was extracted from the database. Per the Centers for Disease Control and Prevention, obesity was defined as body mass index in kg/m^2 of 30 and above.

[a]One hundred two patients had parasitemia data.

group, it is believed that a significant reduction in expression of the cytokines interleukin (IL)-10, and IL-13 in the spleen resulted in more severe disease and duration of infection in coinfected mice [31]. These findings suggest that genetic variation may be a determinant in symptom severity among coinfected individuals. Additionally, in a murine study by Bhanot and Parveen, coinfection with *B. burgdorferi* and *B. microti* attenuated *Babesia* spp. parasite growth while exacerbating Lyme disease symptoms [32]. Another murine model found that the immune activity in response to *Borrelia burgdorferi,* such as increased activation of Th1 and Th17 cells, decreased the *Babesia* parasite burden [33]. A high level of gamma interferon (IFN-γ) produced by $CD4^+$ T cells has been shown to play a key role in the resolution of acute *Babesia* infection and to be involved in protection against other intracellular parasites [34].

Babesiosis has a varying, nonspecific presentation, ranging from asymptomatic infection or mild symptoms to death via multiorgan dysfunction. For example, babesiosis can cause anemia, fever, chills, headache, and sweats, but these presentations can be associated with a plethora of other conditions and, thus, are not specific to babesiosis. Conversely, *Borrelia burgdorferi* has a distinct and well-known temporal symptom profile, including skin, joint, cardiac, and neurological findings. Initial onset of symptoms usually occurs between 1 and 2 weeks after a tick bite in the case of *Borrelia burgdorferi*, which can be earlier than the onset of babesiosis symptoms, which is typically between 1 and 6 weeks following tick bite. As such, in coinfected patients, concern for *Borrelia burgdorferi* could lead to evaluation for tick-borne illnesses, resulting in more prompt diagnosis of babesiosis compared with patients with babesiosis

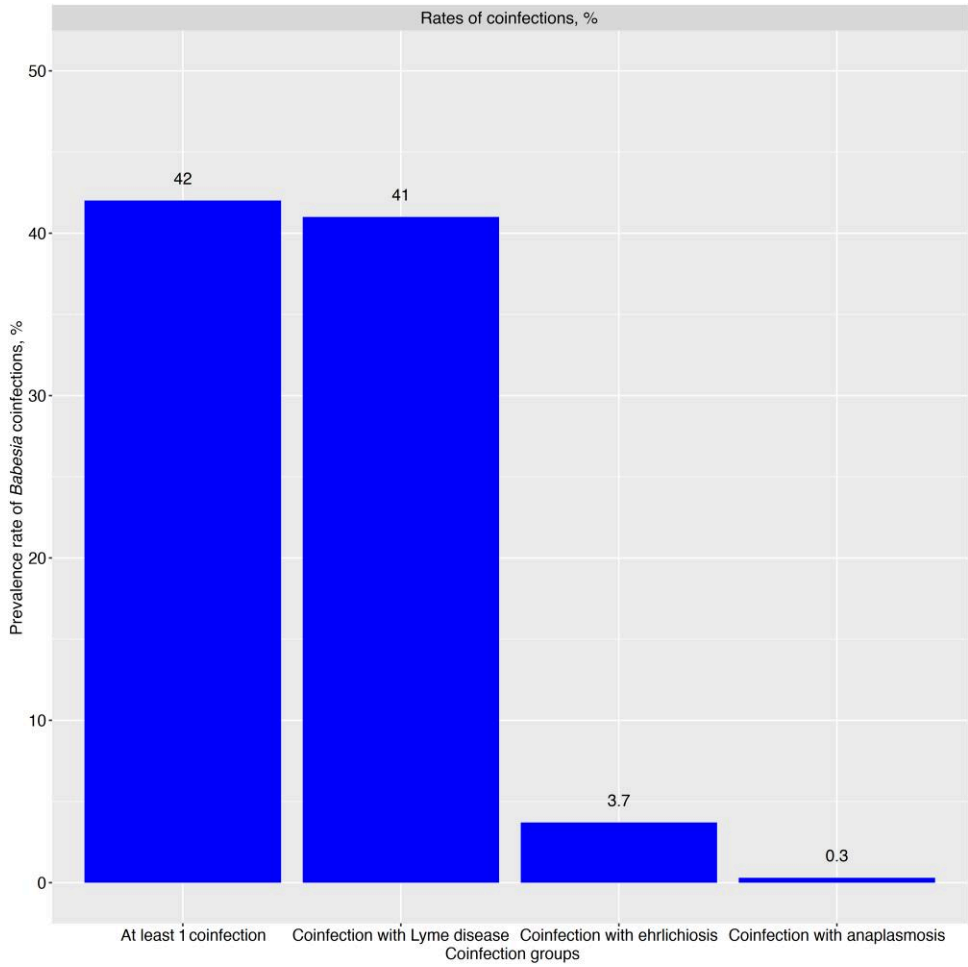

Figure 2. Prevalence of babesiosis coinfections.

alone. This would allow for earlier initiation of treatment in coinfected patients and therefore improve outcomes compared with patients with babesiosis alone, whose diagnosis and treatment might be delayed due to the patients' initial presentation being unclear.

The mortality rate in our cohort was low at 1.4%. In the literature, the mortality rate of babesia ranges from 1.6% to 13% depending on the severity of the disease. In our cohort, ~50% of patients received azithromycin and atovaquone, the mainstay antimicrobial treatment for babesiosis patients. Clindamycin was prescribed in ~15% of the cases, and doxycycline was more likely to be prescribed for the coinfection group than the *Babesia*-only group. The treatment of *Babesia* infection depends on disease severity, with a combination of azithromycin and atovaquone as the preferred treatment for symptomatic individuals with mild to moderate disease [35]. Oral clindamycin and quinine are an alternative option, although they are associated with higher risk of adverse events (including diarrhea, rash, tinnitus, vertigo, and decreased hearing) compared with azithromycin and atovaquone (duration of therapy of 7–10 days) [35]. Severe babesiosis, defined as parasitemia ≥4% (but can also occur with parasitemia <4%), is associated with severe complications including multiple organ dysfunction. Persistent or relapsing disease is treated with intravenous azithromycin plus oral atovaquone or IV clindamycin plus oral quinine as the alternative. Red cell exchange transfusion is reserved for patients with parasitemia >10% or severe organ impairment (such as pulmonary, renal, or hepatic dysfunction) [36]. We did not observe a difference in terms of severe disease between the coinfection and *Babesia*-only patients in our study.

Our findings have potential clinical and public health implications. Health care providers should have a low threshold to examine carefully for an erythema migrans rash or test for other tick-borne confections among hospitalized patients with babesiosis, favoring presumptive treatment for *Borrelia burgdorferi* in this patient population. Therefore, the addition of doxycycline and other anti–*Borrelia burgdorferi* therapy to the most common *Babesia* spp. antimicrobial regimen of

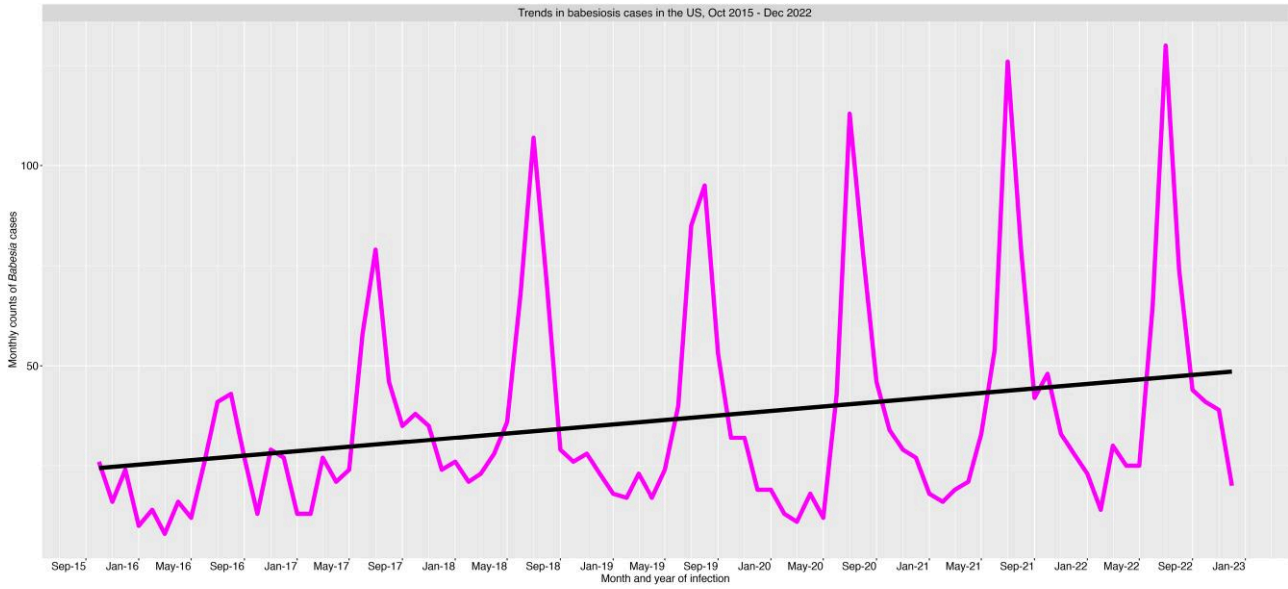

Figure 3. Temporal distribution of babesiosis cases in the United States (2015–2022). Cases peaked in June through September.

Table 2. Multiple Logistic Regression for the Primary Analysis for the Primary Outcome of Association of Coinfection and 90-Day Mortality

Variable	Adjusted Hazard Ratio	95% CI	*P* Value
Coinfection[a]	0.43	0.20–0.92	.03
Age	1.04	1.01–1.07	.003
Sex (male)	1.12	0.59–2.12	.73
Asplenia	2.93	0.92–9.38	.07
Congestive heart failure	1.88	0.87–4.03	.11
Chronic obstructive pulmonary disease	0.94	0.44–2.00	.88
Diabetes	1.03	0.48–2.18	.95
Hypertension	1.24	0.57–2.72	.59
Chronic kidney disease	2.77	1.33–5.80	.007
Lymphoma	2.43	0.90–6.56	.08
Rheumatoid arthritis	0.99	0.40–2.49	.99
Obesity	0.76	0.33–1.79	.53
Depression	0.94	0.43–2.04	.88
Blood loss anemia	1.53	0.48–4.93	.48
Simple blood transfusion	2.86	1.30–6.52	.02

Major confounding variables included in the model were demographics (age, sex), comorbidities (congestive heart failure, chronic obstructive pulmonary disease, diabetes, hypertension, chronic kidney disease, lymphoma, rheumatoid arthritis, obesity, depression), surrogate markers of babesiosis severity (anemia, and blood transfusion), and factors known to influence severe babesiosis (asplenia).

[a]Coinfection was defined as babesiosis with 1 or more additional tick-borne infections: Lyme disease, anaplasmosis, or ehrlichiosis. Effect estimates of the confounding variables are also reported in the table to show other important clinical variables that could be associated with mortality in babesiosis populations.

atovaquone and azithromycin could facilitate improved outcomes. It is important to note that doxycycline also has both in vitro and in vivo activity against *Babesia gibsoni* and *Babesia canis*; however, activity against human babesiosis has only been described in isolated case reports [37–40]. To date, no trials with doxycycline have been conducted in human patients with *Babesia* infections. Conversely, *Borrelia burgdorferi* laboratory testing usually consists of Lyme disease antibody testing. This test provides limited sensitivity and specificity because the presence of antibodies may be delayed for several weeks after the onset of acute disease, and the presence of antibodies may be due to a previous infection. Thus, testing everyone who has babesiosis for Lyme disease would probably not be cost-effective and would create both false-positive and false-negative results. Lyme disease antibody testing might be more cost-effective for those who do not have erythema migrans rash but have clinical findings suggestive of Lyme disease, such as arthritis, carditis, or meningitis. Selective laboratory testing for other coinfections would also be appropriate in those with persistent symptoms despite anti-*Babesia* antimicrobial agents. Furthermore, coinfection of babesiosis patients, other than those with Lyme disease, is uncommon. For example, Powassan coinfection of babesiosis patients is very infrequent, and laboratory testing is not generally available. Laboratory testing for Powassan infection in babesiosis patients would be reserved for those with signs and symptoms of encephalitis.

Our study has several strengths, including the large sample size using real-world data and the inclusion of patients from most regions of the United States, particularly regions where *Babesia* is endemic or an emerging infection. Due to the large sample size, the study had adequate power to adjust for multiple potential confounding factors for the association between coinfecting tick-borne zoonoses and severe disease. However, the findings of the present study should be interpreted in light

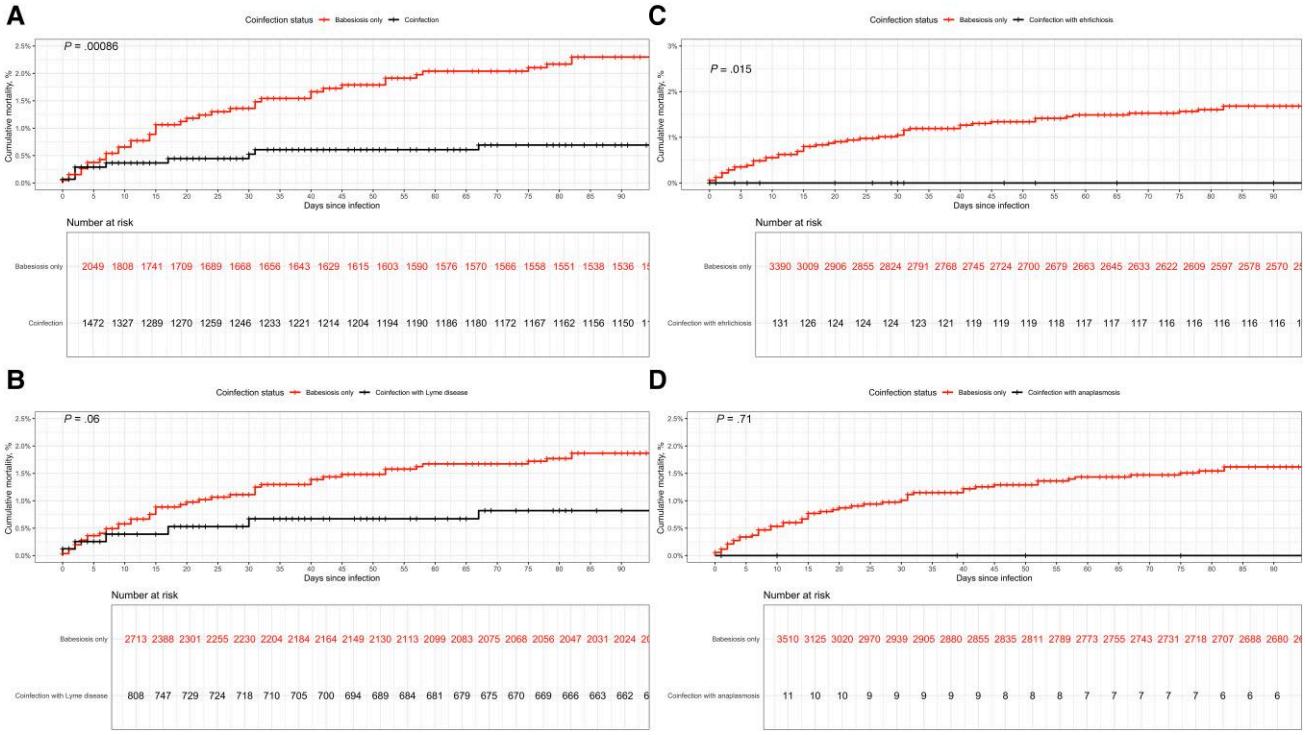

Figure 4. Cumulative incidence graphs showing the association of coinfection and 90-day mortality for overall coinfection (A), coinfection with *Borrelia burgdorferi* (B), coinfection with ehrlichiosis (C), and coinfection with anaplasmosis (D).

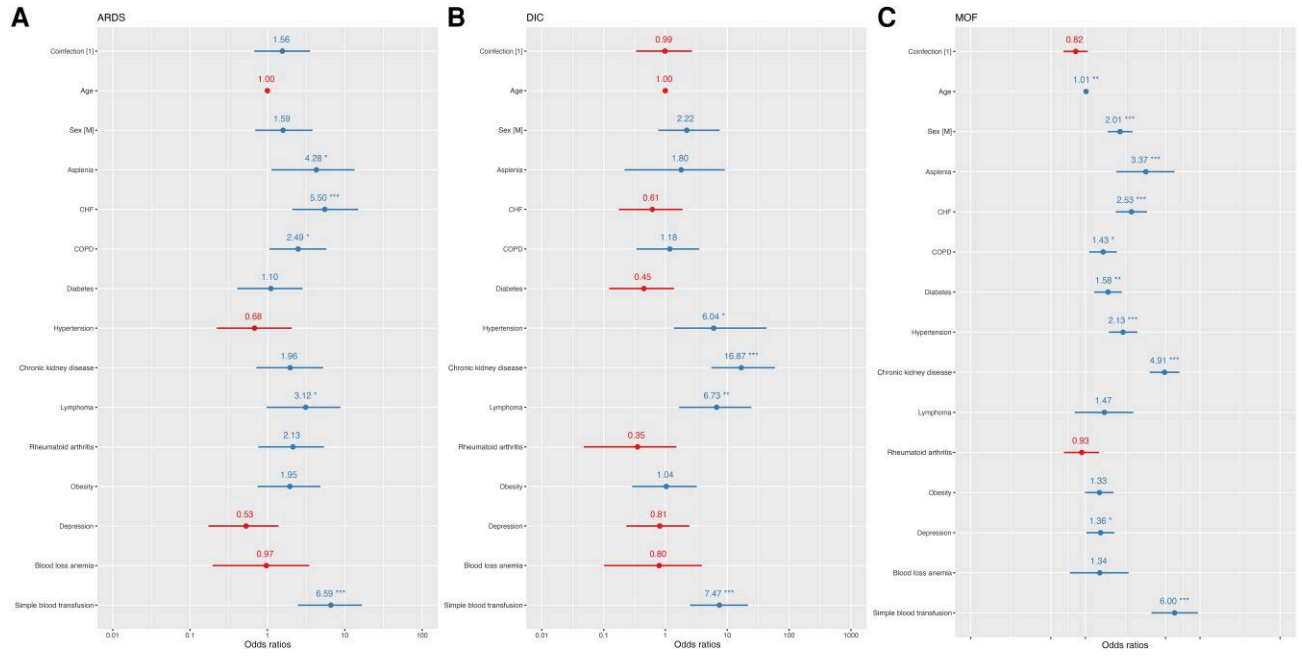

Figure 5. Association of coinfection with secondary outcomes from multivariable logistic regression models. A, Acute respiratory distress syndrome. B, Disseminated intravascular coagulopathy. C, Multiorgan failure. Covariates adjusted in the model include demographics (age, sex), comorbidities (congestive heart failure, chronic obstructive pulmonary disease, diabetes, hypertension, chronic kidney disease, lymphoma, rheumatoid arthritis, obesity, depression), surrogate markers of babesiosis severity (anemia and blood transfusion), and factors known to influence severe babesiosis (asplenia). Abbreviations: ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; CHF, congestive heart failure; DIC, disseminated intravascular coagulopathy; MOF, multiorgan failure.

of some limitations. Although we adjusted for major confounding factors in the multivariable logistic regression models, we did not adjust for parasite burden. We were unable to find adequate parasitemia-level data in the TriNetX data set as just a few patients had these data available; the data were therefore not adequate for subgroup analysis. However, our statistical models included biomarkers of severe *Babesia* disease, such as anemia and the need for blood transfusion, which were surrogate biomarkers of severe babesiosis in the absence of parasitemia level. Additionally, it is plausible that there was residual confounding induced by comorbidities not included in the models.

CONCLUSIONS

In this extensive study of >3000 patients with babesiosis in the United States, the prevalence of coinfection was highest with *Borrelia burgdorferi*, followed by ehrlichiosis, and lowest with anaplasmosis. This study does not support our hypothesis that *Babesia* coinfection with other tick-borne pathogens is associated with higher severity of disease and higher mortality risk. Future studies are needed to investigate possible therapeutic benefit of doxycycline in babesiosis as to date no trials with doxycycline have been conducted in human patients with *Babesia* infections.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. Dr. Ssentongo had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Dr. Ssentongo and Dr. Venugopal contributed equally as first authors. Concept and study design: Ssentongo. Acquisition of data from database: Ba, Zhang. Statistical analysis: Ssentongo, Chinchilli. Drafting of the manuscript: Ssentongo, Venugopal. Critical revision of the manuscript for important intellectual content: all authors. Obtained funding: Ssentongo.

Role of the funder/sponsor. The funding organization had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Additional information. To facilitate replication of these findings, R code and data to reproduce the results in this article are archived at GitHub. The link to the GitHub code and data is https://github.com/ssentongojeddy/Babesia_Coinfection/tree/main.

Patient consent. Data are from the TriNetX database, a federated network, and received a waiver from the Western IRB as only aggregated counts and de-identified information were used. Additionally, the protocol of this study was reviewed and received a determination of non–human subjects research by the Penn State Institutional Review Board. The individual informed consent requirement was waived for this secondary analysis of de-identified data.

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Potential conflicts of interest. All authors: no conflicts of interest to disclose.

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